abcam

Product datasheet

Anti-PD-L1 antibody [EPR19759] - BSA and Azide free ab221612

敲除验证 重组 RabMAb

1 Abreviews 14 图像

概述

产品名称	Anti-PD-L1抗体[EPR19759] - BSA and Azide free
描述	兔单克隆抗体[EPR19759] to PD-L1 - BSA and Azide free
宿主	Rabbit
经测试应用	**适用于:** ICC/IF, IHC-P, IP, WB, Flow Cyt (Intra)
种属反应性	**与反应:** Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. (Peptide available as **ab150077**)
阳性对照	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.
常规说明	ab221612 is the carrier-free version of **ab213524**. 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. 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**.

性能

形式	Liquid
存放说明	Shipped at 4°C. Store at +4°C. Do Not Freeze.
存储溶液	pH: 7.2 Constituent: PBS
无载体	**是**
纯度	Protein A purified
克隆	单克隆
克隆编号	EPR19759
同种型	IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用ab221612于以下的经测试应用**

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

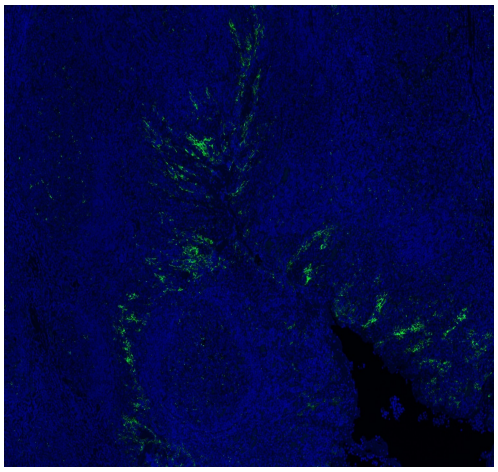
应用	Ab评论	说明
ICC/IF		Use at an assay dependent concentration.
IHC-P	★★★☆☆ (1)	Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. Antigen retrieval: Universal HIER antigen retrieval reagent (**ab208572**).
IP		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 40-45 kDa (predicted molecular weight: 33 kDa).Can be blocked with **Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077)**.
Flow Cyt (Intra)		Use at an assay dependent concentration.

靶标

功能	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.
组织特异性	Highly expressed in the heart, skeletal muscle, placenta and lung. Weakly expressed in the

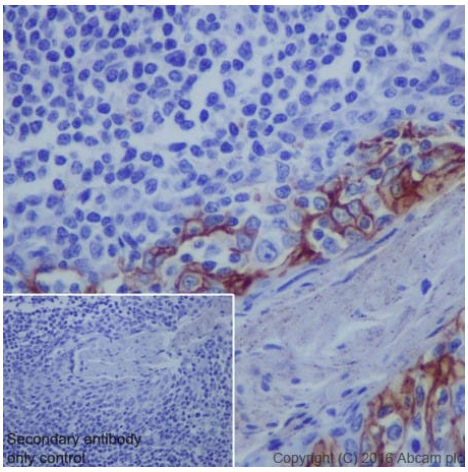
	thymus, spleen, kidney and liver. Expressed on activated T- and B-cells, dendritic cells, keratinocytes and monocytes.
序列相似性	Belongs to the immunoglobulin superfamily. BTN/MOG family. Contains 1 Ig-like C2-type (immunoglobulin-like) domain. Contains 1 Ig-like V-type (immunoglobulin-like) domain.
细胞定位	Cell membrane and Endomembrane system.

图片



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [EPR19759] - BSA and Azide free (ab221612)

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] - BSA and Azide free (ab221612)

This IHC data was generated using the same anti-PDL1 antibody clone, EPR19759, in a different buffer formulation (cat# **ab213524**).

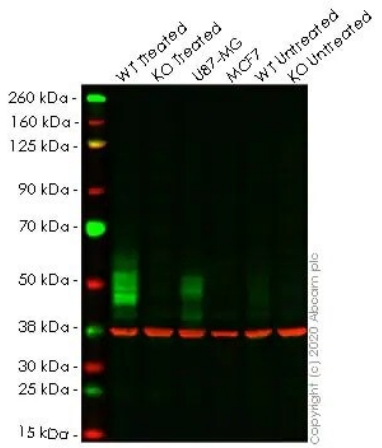
Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling PD-L1 with **ab213524** at 1/250 dilution, followed by Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

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**).

Heat mediated antigen retrieval was performed before commencing with IHC staining protocol.



Western blot - Anti-PD-L1 antibody [EPR19759] - BSA and Azide free (ab221612)

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
Lane 2 : 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
Lane 6 : CD274 knockout A549 untreated cell lysate

Lysates/proteins at 20 µg per lane.

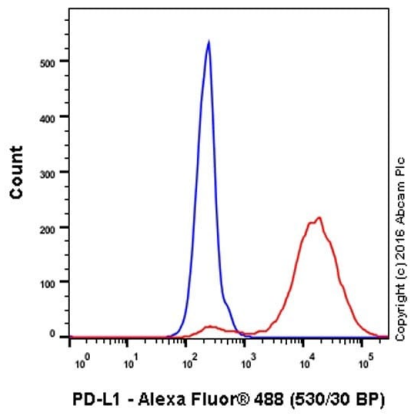
Performed under reducing conditions.

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

This data was developed using the same antibody clone in a different buffer formulation (**ab213524**).

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

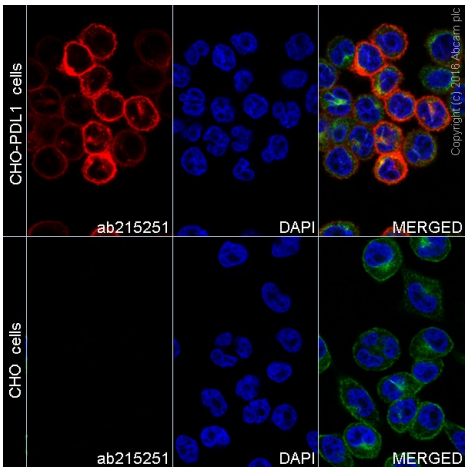
ab213524 Recombinant Anti-PD-L1 antibody [EPR19759] was shown to specifically react with PD-L1 in wild-type A549 treated with 100 ng/mL IFN gamma for 48 h cells in western blot. Loss of signal was observed when both treated and untreated knockout cell line **ab267055** (treated and untreated knockout 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.



Flow Cytometry (Intracellular) - Anti-PD-L1 antibody [EPR19759] - BSA and Azide free (ab221612)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab213524**).



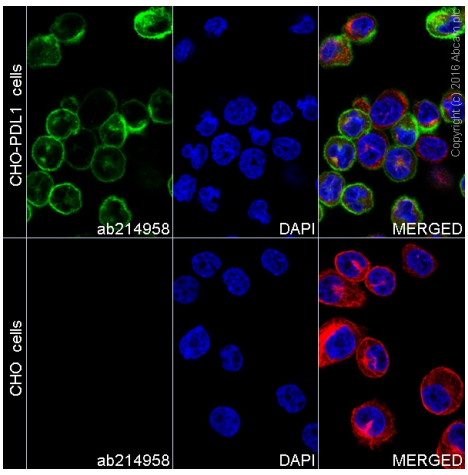
Immunocytochemistry/ Immunofluorescence - Anti-PD-L1 antibody [EPR19759] - BSA and Azide free (ab221612)

Clone EPR19759 (ab221612) has been successfully conjugated by Abcam. This image was generated using Anti-PD-L1 antibody [EPR19759] (Alexa Fluor® 647). Please refer to **ab215251** for protocol details.

ab215251 staining PDL1 in CHO-PDL1 cells. The lower panels demonstrate that **ab215251** does not cross react with un-transfected CHO cells.

The cells were fixed with 4% formaldehyde (10 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with **ab215251** at 1/200 dilution (shown in red) and **ab195887**, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 488), at 1/250 dilution (shown in green). Nuclear DNA was labelled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



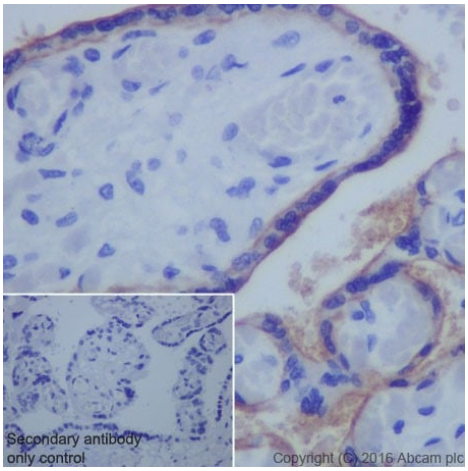
Immunocytochemistry/ Immunofluorescence - Anti-PD-L1 antibody [EPR19759] - BSA and Azide free (ab221612)

Clone EPR19759 (ab221612) has been successfully conjugated by Abcam. This image was generated using Anti-PD-L1 antibody [EPR19759] (Alexa Fluor® 488). Please refer to **ab214958** for protocol details.

ab214958 staining PDL1 in CHO-PDL1 cells. The lower panels demonstrate that **ab214958** does not cross react with un-transfected CHO cells.

The cells were fixed with 4% formaldehyde (10 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with **ab214958** at 1/200 dilution (shown in green) and **ab195889**, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 594), at 1/250 dilution (shown in red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [EPR19759] - BSA and Azide free (ab221612)

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**).

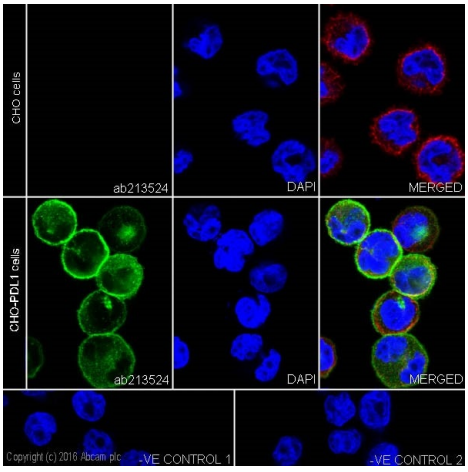
Membrane staining on the human placenta is observed.

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**)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab213524**).

Heat mediated antigen retrieval was performed before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-PD-L1 antibody [EPR19759] - BSA and Azide free (ab221612)

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).

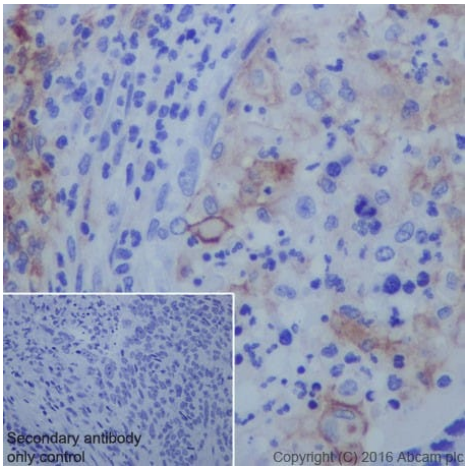
Tubulin is detected with Anti-alpha Tubulin mouse MAb (**ab7291**) at 1/1000 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (**ab150120**) secondary antibody at 1/1000 dilution (red).

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab213524**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [EPR19759] - BSA and Azide free (ab221612)

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**).

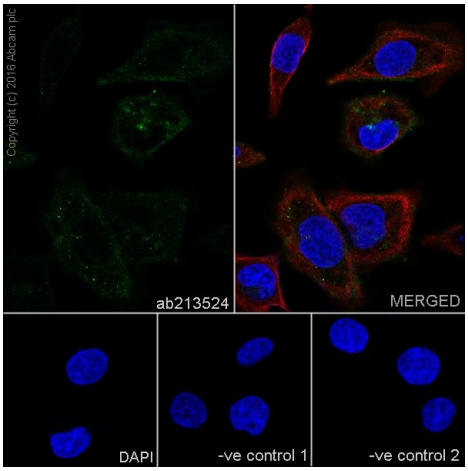
Membrane staining on the human stomach cancer is observed.

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**).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab213524**).

Heat mediated antigen retrieval was performed before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-PD-L1 antibody [EPR19759] - BSA and Azide free (ab221612)

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).

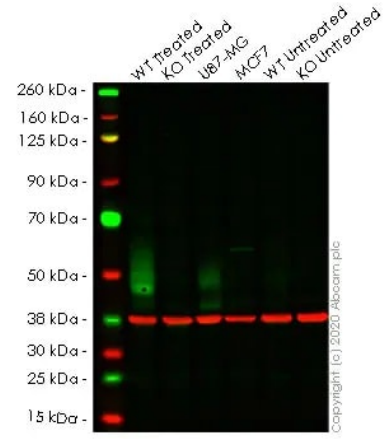
Tubulin is detected with Anti-alpha Tubulin mouse MAb (**ab7291**) at 1/1000 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (**ab150120**) secondary antibody at 1/1000 dilution (red).

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab213524**).



Western blot - Anti-PD-L1 antibody [EPR19759] - BSA and Azide free (ab221612)

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.

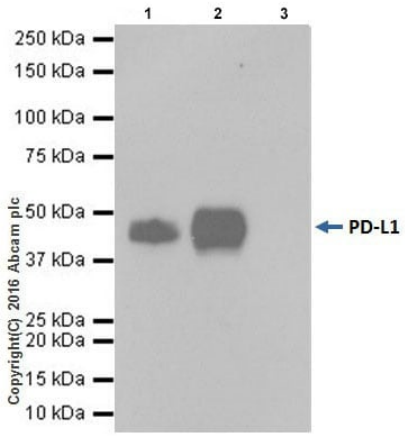
Performed under reducing conditions.

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

This data was developed using the same antibody clone in a different buffer formulation (**ab213524**).

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.



Immunoprecipitation - Anti-PD-L1 antibody [EPR19759] - BSA and Azide free (ab221612)

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 1/10000 dilution.

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

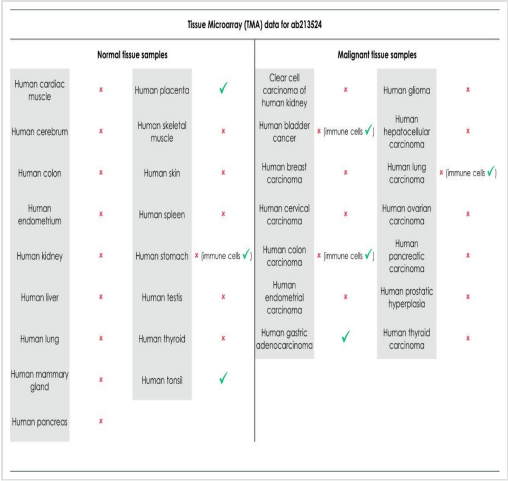
Lane 2: **ab213524** IP in NCI-H1975 whole cell lysate.

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab213524**).



Tissue Microarray (TMA) data for ab213524

Normal tissue samples

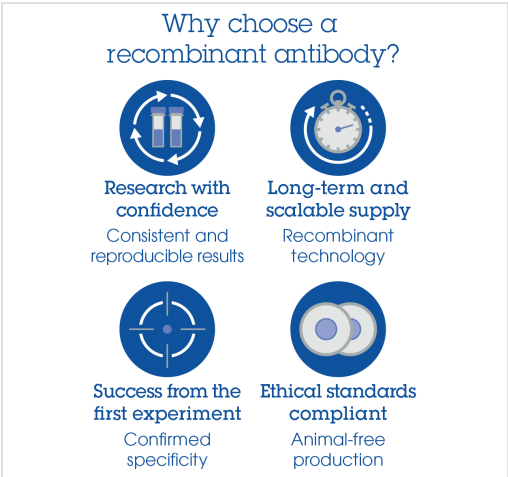
Human cardiac muscle	x	Human placenta	✓		
Human cerebrum	x	Human skeletal muscle	x		
Human colon	x	Human skin	x		
Human endometrium	x	Human spleen	x		
Human kidney	x	Human stomach	x (immune cells ✓)		
Human liver	x	Human testis	x		
Human lung	x	Human thyroid	x		
Human mammary gland	x	Human tonsil	✓		
Human pancreas	x				

Malignant tissue samples

Clear cell carcinoma of human kidney	x	Human glioma	x
Human bladder cancer	x (immune cells ✓)	Human hepatocellular carcinoma	x
Human breast carcinoma	x	Human lung carcinoma	x (immune cells ✓)
Human cervical carcinoma	x	Human ovarian carcinoma	x
Human colon carcinoma	x (immune cells ✓)	Human pancreatic carcinoma	x
Human endometrial carcinoma	x	Human prostatic hyperplasia	x
Human gastric adenocarcinoma	✓	Human thyroid carcinoma	x

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD-L1 antibody [EPR19759] - BSA and Azide free (ab221612)

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] - BSA and Azide free (ab221612)

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

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours

- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise,

please visit **https://www.abcam.cn/abpromise** or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors