abcam

Product datasheet

Anti-PD1 antibody [CAL20] ab237728

Recombinant RabMAb

1 Abreviews 6 References 15 Images

Overview

Product name	Anti-PD1 antibody [CAL20]
Description	Rabbit monoclonal [CAL20] to PD1
Host species	Rabbit
Tested applications	**Suitable for:** IHC-P, mIHC, WB, ICC/IF **Unsuitable for:** Flow Cyt or IP
Species reactivity	**Reacts with:** Human, Rhesus monkey
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: MOLT-4 (treated with 10ng/ml PMA and 500ng/ml Ionomycin for 24h) whole cell lysate. ICC/IF: MOLT-4 cells (treated with 10ng/ml PMA and 500ng/ml Ionomycin for 24h). IHC-P: Human tonsil, lymph node and lung carcinoma tissues. mIHC: Human tonsil tissue and Human breast cancer tissue.

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: 0.05% Sodium azide Constituent: PBS
Purity	Protein A purified
Purification notes	Purity >99%
Clonality	Monoclonal
Clone number	CAL20
Isotype	IgG

Applications

The Abpromise guarantee	Our **Abpromise guarantee** covers the use of ab237728 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	★★★★★ (1)	1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
mIHC		Use at an assay dependent concentration.
WB		1/1000. Detects a band of approximately 50-55 kDa (predicted molecular weight: 32 kDa).
ICC/IF		1/50.

Application notes Is unsuitable for Flow Cyt or IP.

Target

Function Possible cell death inducer, in association with other factors.

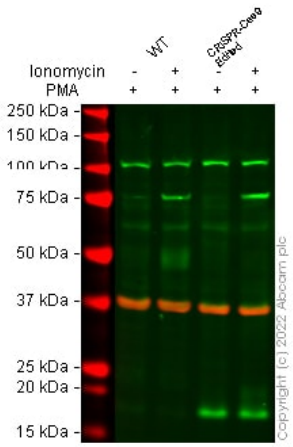
Involvement in disease Genetic variation in PDCD1 is associated with susceptibility to systemic lupus erythematosus type 2 (SLEB2) [MIM:605218]. Systemic lupus erythematosus is a chronic, inflammatory and often febrile multisystemic disorder of connective tissue. It affects principally the skin, joints, kidneys and serosal membranes. It is thought to represent a failure of the regulatory mechanisms of the autoimmune system.

Sequence similarities Contains 1 Ig-like V-type (immunoglobulin-like) domain.

Developmental stage Induced at programmed cell death.

Cellular localization Membrane.

Images



Western blot - Anti-PD1 antibody [CAL20] (ab237728)

All lanes : Anti-PD1 antibody [CAL20] (ab237728) at 1/500 dilution

Lane 1 : Wild-type HeLa Vehicle Control Ionomycin (0 ng/mL, 24h) + PMA (10 ng/mL, 24 h) cell lysate

Lane 2 : Wild-type HeLa Treated Ionomycin (500 ng/mL, 24h) + PMA (10 ng/mL, 24 h) cell lysate

Lane 3 : PDCD1 knockout Hela vehicle Control Ionomycin(0ng/mL 24h)+PMA(10ng/mL 24h) cell lysate

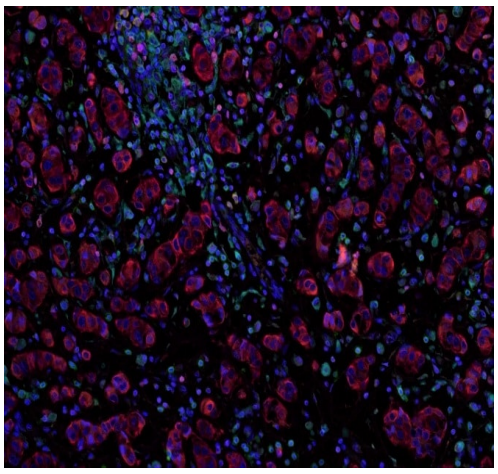
Lane 4 : PDCD1 knockout Hela Ionomycin(500ng/mL 24h)+PMA(10ng/mL 24h) cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 32 kDa
Observed band size: 48 kDa

False colour image of Western blot: Anti-PD1 antibody [CAL20] staining at 1/500 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab237728 was shown to bind specifically to PD1. A band was observed at 48 kDa in wild-type HeLa cell lysates with no signal observed at this size in PDCD1 CRISPR-Cas9 edited cell line **ab255417** (CRISPR-Cas9 edited cell lysate **ab263794**). The band observed in the CRISPR-Cas9 edited lysate lane below 48 kDa is likely to represent a truncated form of PD1. This has not been investigated further and the functional properties of the gene product have not been determined. To generate this image, wild-type and PDCD1 CRISPR-Cas9 edited HeLa 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-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.



Multiplex immunohistochemistry - Anti-PD1 antibody [CAL20] (ab237728)

This image is courtesy of ImmunoAtlas.

Fluorescence multiplex immunohistochemical analysis of Human breast cancer tissue (formalin-fixed paraffin-embedded section).

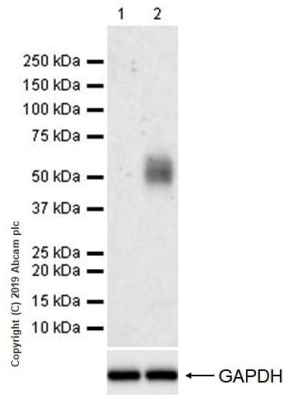
Merged staining of Anti-PD-L1 (**ab251611**; cyan; Opal™ 520), Anti-Granzyme B (**ab219803**; yellow; Opal™ 540), Anti-PD1 (**ab251613**; magenta; Opal™ 570), Anti-pan Cytokeratin (**ab264485**; red; Opal™ 620), Anti-EpCAM (**ab225894**; red; Opal™ 620), Anti-CD8 alpha (**ab251596**; green; Opal™ 650) and Anti-FOXP3 (**ab96048**; orange; Opal™ 690). EpCAM and pan-cytokeratin share the same dye and color. Dyes are pseudo-colored for better contrast of the markers.

The immunostaining was performed on a Leica Biosystems BOND® MAX instrument with an Opal™ 6-Plex Detection Kit (NEL821001KT, Akoya Biosciences®).

The section was incubated in six rounds of staining; sequentially for **ab251611** (1/750 dilution), **ab219803** (1/250 dilution), **ab251613** (1/750 dilution), **ab264485** (0.5 µg/ml), **ab225894** (1/1250 dilution),

ab251596 (1/1500 dilution) and **ab96048** (10 µg/ml); each using a separate fluorescent tyramide signal amplification system. EDTA based antigen retrieval (Leica Biosystems BOND® Epitope Retrieval Solution 2, pH 9.0, 20 minutes) was used in between rounds of tyramide signal amplification to remove the antibody from the previous round, to avoid any cross-reactivity. DAPI (dark blue) was used as a nuclear counter stain.
Microscopy and pseudocoloring of individual Opal™ dyes was performed using a Vectra 3 Imaging System (Akoya Biosciences®).

This data is courtesy of ImmunoAtlas and it can be found **here**.



Western blot - Anti-PD1 antibody [CAL20] (ab237728)

All lanes : Anti-PD1 antibody [CAL20] (ab237728) at 1/1000 dilution

Lane 1 : Untreated MOLT-4 (human lymphoblastic leukemia cell line), whole cell lysate
Lane 2 : MOLT-4 (treated with 10ng/ml PMA and 500ng/ml Ionomycin for 24h), whole cell lysate

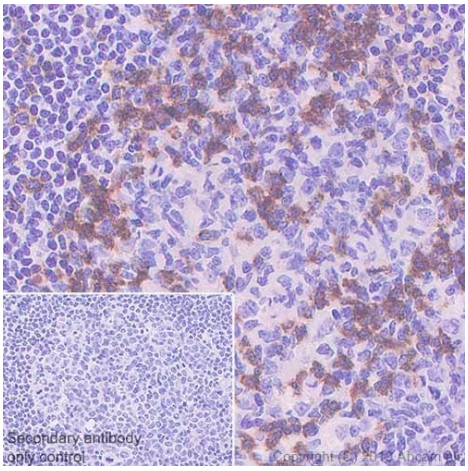
Lysates/proteins at 20 µg per lane.

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

Predicted band size: 32 kDa
Observed band size: 50-55 kDa

Exposure time: 3 minutes

Blocking and dilution buffer: 5% NFDM/TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD1 antibody [CAL20] (ab237728)

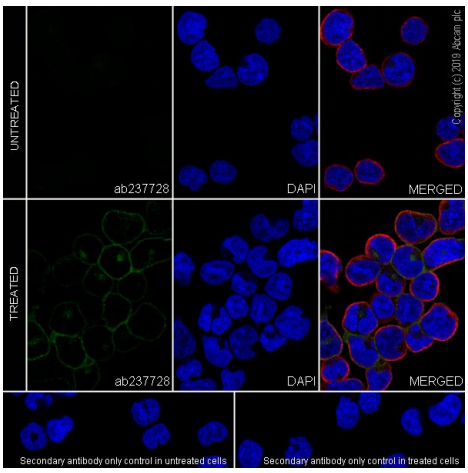
Immunohistochemical analysis of paraffin-embeded human tonsil tissue labeling PD1 with ab237728 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) ready to use. Positive staining on the human tonsil.is observed. Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) ready to use.

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins.

The section was incubated with ab237728 for 15 mins at room temperature.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument.

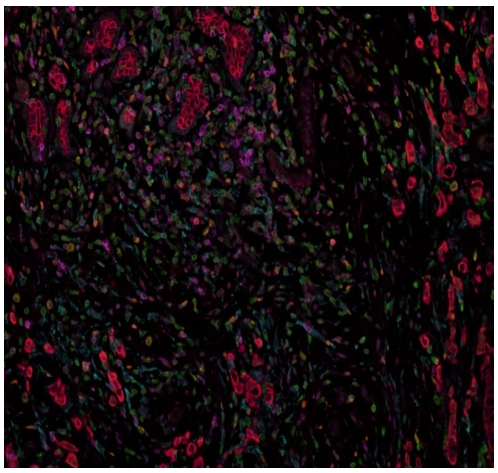


Immunocytochemistry/ Immunofluorescence - Anti-PD1 antibody [CAL20] (ab237728)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized MOLT-4 (human lymphoblastic leukemia cell line) cells labeling PD1 with ab237728 at 1/50 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing membranous staining in MOLT-4 cells treated with PMA (10ng/ml 24h) and Ionomycin(500ng/ml 24h). The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (**ab195889**) at 1/200 dilution (red).

Secondary antibody only control: Used PBS instead of primary antibody, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution.

Cells were either untreated, ot treated with treated with PMA (10ng/ml 24h) and Ionomycin (500ng/ml 24h).



Multiplex immunohistochemistry - Anti-PD1 antibody [CAL20] (ab237728)

This image is courtesy of ImmunoAtlas.

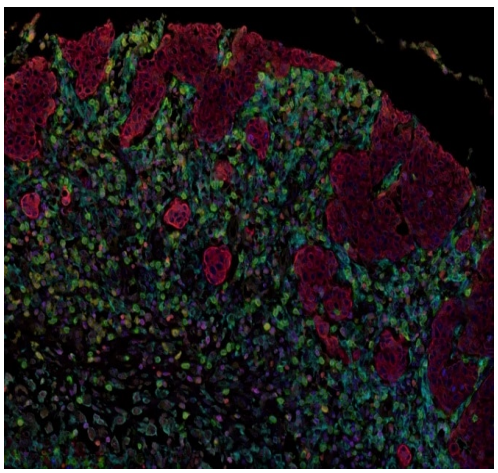
Fluorescence multiplex immunohistochemical analysis of Human breast cancer tissue (formalin-fixed paraffin-embedded section).

Merged staining of Anti-PD-L1 (**ab251611**; cyan; Opal™ 520), Anti-Granzyme B (**ab219803**; yellow; Opal™ 540), Anti-PD1 (**ab251613**; magenta; Opal™ 570), Anti-pan Cytokeratin (**ab264485**; red; Opal™ 620), Anti-EpCAM (**ab225894**; red; Opal™ 620), Anti-CD8 alpha (**ab251596**; green; Opal™ 650) and Anti-FOXP3 (**ab96048**; orange; Opal™ 690). EpCAM and pan-cytokeratin share the same dye and color. Dyes are pseudo-colored for better contrast of the markers.

The immunostaining was performed on a Leica Biosystems BOND® MAX instrument with an Opal™ 6-Plex Detection Kit (NEL821001KT, Akoya Biosciences®).

The section was incubated in six rounds of staining; sequentially for **ab251611** (1/750 dilution), **ab219803** (1/250 dilution), **ab251613** (1/750 dilution), **ab264485** (0.5 µg/ml), **ab225894** (1/1250 dilution), **ab251596** (1/1500 dilution) and **ab96048** (10 µg/ml); each using a separate fluorescent tyramide signal amplification system. EDTA based antigen retrieval (Leica Biosystems BOND® Epitope Retrieval Solution 2, pH 9.0, 20 minutes) was used in between rounds of tyramide signal amplification to remove the antibody from the previous round, to avoid any cross-reactivity. DAPI (dark blue) was used as a nuclear counter stain.
Microscopy and pseudocoloring of individual Opal™ dyes was performed using a Vectra 3 Imaging System (Akoya Biosciences®).

This data is courtesy of ImmunoAtlas and it can be found **here**.



Multiplex immunohistochemistry - Anti-PD1 antibody [CAL20] (ab237728)

This image is courtesy of ImmunoAtlas.

Fluorescence multiplex immunohistochemical analysis of Human breast cancer tissue (formalin-fixed paraffin-embedded section).

Merged staining of Anti-PD-L1 (**ab251611**; cyan; Opal™ 520), Anti-Granzyme B (**ab219803**; yellow; Opal™ 540), Anti-PD1 (**ab251613**; magenta; Opal™ 570), Anti-pan Cytokeratin (**ab264485**; red; Opal™ 620), Anti-EpCAM (**ab225894**; red; Opal™ 620), Anti-CD8 alpha (**ab251596**; green; Opal™ 650) and Anti-FOXP3 (**ab96048**; orange; Opal™ 690). EpCAM and pan-cytokeratin share the same dye and color. Dyes are pseudo-colored for better contrast of the markers.

The immunostaining was performed on a Leica Biosystems BOND® MAX instrument with an Opal™ 6-Plex Detection Kit (NEL821001KT, Akoya Biosciences®).

The section was incubated in six rounds of staining; sequentially for **ab251611** (1/750 dilution), **ab219803** (1/250 dilution), **ab251613** (1/750 dilution), **ab264485** (0.5 µg/ml), **ab225894** (1/1250 dilution), **ab251596** (1/1500 dilution) and **ab96048** (10 µg/ml); each using a separate fluorescent tyramide signal amplification system. EDTA based antigen retrieval (Leica Biosystems BOND® Epitope Retrieval Solution 2, pH 9.0, 20 minutes) was used in between rounds of tyramide signal amplification to remove the antibody from the previous round, to avoid any cross-reactivity. DAPI (dark blue) was used as a nuclear counter stain.
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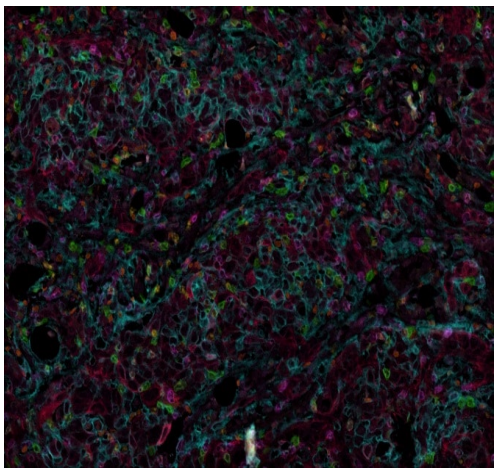

Multiplex immunohistochemistry - Anti-PD1 antibody [CAL20] (ab237728)

This image is courtesy of ImmunoAtlas.

Fluorescence multiplex immunohistochemical analysis of Human breast cancer tissue (formalin-fixed paraffin-embedded section).

Merged staining of Anti-PD-L1 (**ab251611**; cyan; Opal™ 520), Anti-Granzyme B (**ab219803**; yellow; Opal™ 540), Anti-PD1 (**ab251613**; magenta; Opal™ 570), Anti-pan Cytokeratin (**ab264485**; red; Opal™ 620), Anti-EpCAM (**ab225894**; red; Opal™ 620), Anti-CD8 alpha (**ab251596**; green; Opal™ 650) and Anti-FOXP3 (**ab96048**; orange; Opal™ 690). EpCAM and pan-cytokeratin share the same dye and color. Dyes are pseudo-colored for better contrast of the markers.

The immunostaining was performed on a Leica Biosystems BOND® MAX instrument with an Opal™ 6-Plex Detection Kit (NEL821001KT, Akoya Biosciences®).

The section was incubated in six rounds of staining; sequentially for **ab251611** (1/750 dilution), **ab219803** (1/250 dilution), **ab251613** (1/750 dilution), **ab264485** (0.5 µg/ml), **ab225894** (1/1250 dilution), **ab251596** (1/1500 dilution) and **ab96048** (10 µg/ml); each using a separate fluorescent tyramide signal amplification system. EDTA based antigen retrieval (Leica Biosystems BOND® Epitope Retrieval Solution 2, pH 9.0, 20 minutes) was used in between rounds of tyramide signal amplification to remove the antibody from the previous round, to avoid any cross-reactivity. DAPI (dark blue) was used as a nuclear counter stain.
Microscopy and pseudocoloring of individual Opal™ dyes was performed using a Vectra 3 Imaging System (Akoya Biosciences®).

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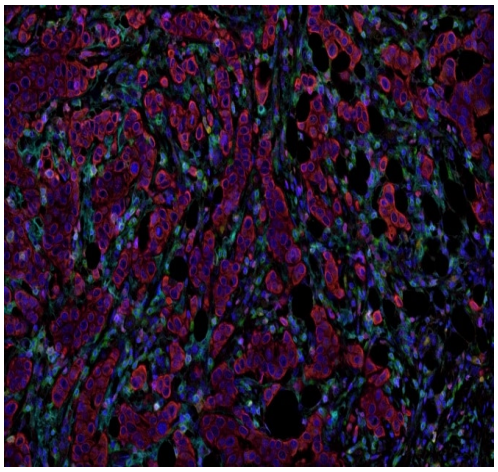


Multiplex immunohistochemistry - Anti-PD1 antibody [CAL20] (ab237728)

This image is courtesy of ImmunoAtlas.

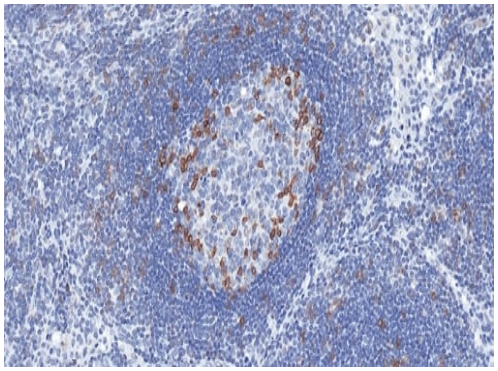
Fluorescence multiplex immunohistochemical analysis of Human breast cancer tissue (formalin-fixed paraffin-embedded section).

Merged staining of Anti-PD-L1 (**ab251611**; cyan; Opal™ 520), Anti-Granzyme B (**ab219803**; yellow; Opal™ 540), Anti-PD1 (**ab251613**; magenta; Opal™ 570), Anti-pan Cytokeratin (**ab264485**; red; Opal™ 620), Anti-EpCAM (**ab225894**; red; Opal™ 620), Anti-CD8 alpha (**ab251596**; green; Opal™ 650) and Anti-FOXP3 (**ab96048**; orange; Opal™ 690). EpCAM and pan-cytokeratin share the same dye and color. Dyes are pseudo-colored for better contrast of the markers.

The immunostaining was performed on a Leica Biosystems BOND® MAX instrument with an Opal™ 6-Plex Detection Kit (NEL821001KT, Akoya Biosciences®).

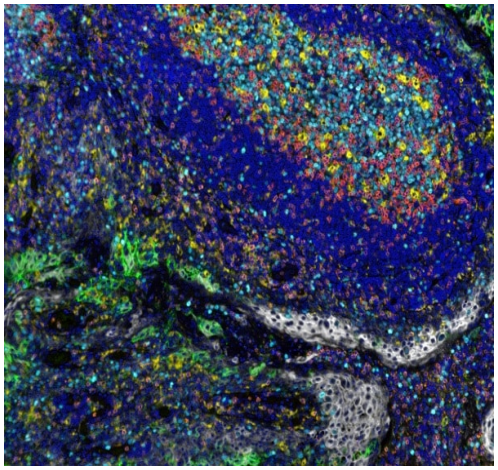
The section was incubated in six rounds of staining; sequentially for **ab251611** (1/750 dilution), **ab219803** (1/250 dilution), **ab251613** (1/750 dilution), **ab264485** (0.5 µg/ml), **ab225894** (1/1250 dilution), **ab251596** (1/1500 dilution) and **ab96048** (10 µg/ml); each using a separate fluorescent tyramide signal amplification system. EDTA based antigen retrieval (Leica Biosystems BOND® Epitope Retrieval Solution 2, pH 9.0, 20 minutes) was used in between rounds of tyramide signal amplification to remove the antibody from the previous round, to avoid any cross-reactivity. DAPI (dark blue) was used as a nuclear counter stain.
Microscopy and pseudocoloring of individual Opal™ dyes was performed using a Vectra 3 Imaging System (Akoya Biosciences®).

This data is courtesy of ImmunoAtlas and it can be found **here**.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD1 antibody [CAL20] (ab237728)

Formalin-fixed, paraffin-embedded human lymph node tissue stained for PD1 using ab237728 at 0.125 µg/ml dilution in immunohistochemical analysis.



Multiplex immunohistochemistry - Anti-PD1 antibody [CAL20] (ab237728)

Fluorescence multiplex immunohistochemical analysis of normal human tonsil tissue (formalin-fixed paraffin-embedded section).

Merged staining of anti-PD1 (ab237728; orange; Opal™520), anti-PDL1 (**ab237726**; green; Opal™540), anti-CD68 (**ab192847**; yellow; Opal™570), anti-CD3 (**ab16669**; red; Opal™620), anti-Ki67 (**ab16667**; light blue; Opal™650) and anti-PanCK (**ab7753**; grey; Opal™690).

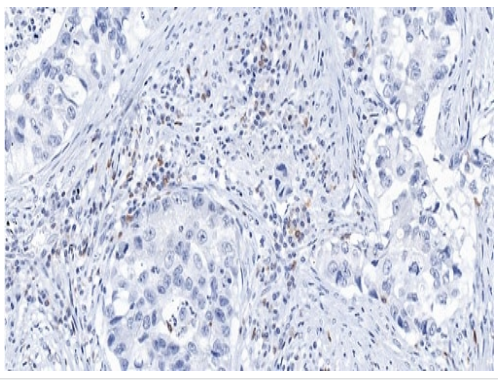
The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 7-color automation IHC kit (NEL821001KT, Akoya Biosciences®).

The section was incubated in six rounds of staining; in the order of ab237728 (1/500 dilution), **ab237726** (1/500 dilution), **ab192847** (1/300 dilution), **ab16669** (1/300 dilution), **ab16667** (1/200 dilution) and **ab7753** (1/200 dilution); each using a separate fluorescent tyramide signal amplification system.

Sodium citrate antigen retrieval (Leica ER1, pH6.0, 30 minutes) was used in between rounds of tyramide signal amplification to remove the antibody from the previous round, to avoid any cross-reactivity.

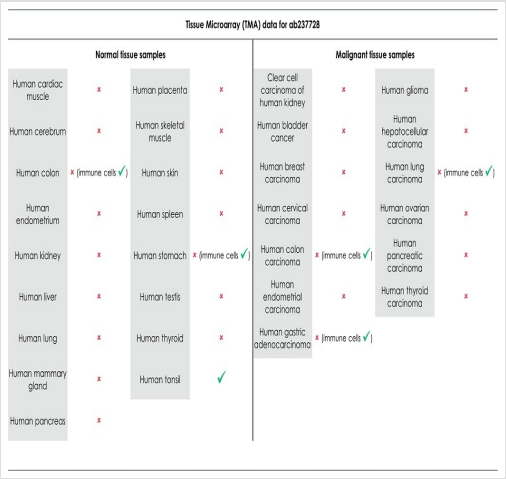
DAPI (dark blue) was used as a nuclear counter stain.

Microscopy and pseudocoloring of individual Opal™ dyes was performed using a Vectra Polaris.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD1 antibody [CAL20] (ab237728)

Formalin-fixed, paraffin-embedded human lung carcinoma tissue stained for PD1 using ab237728 at 0.125 µg/ml dilution in immunohistochemical analysis.

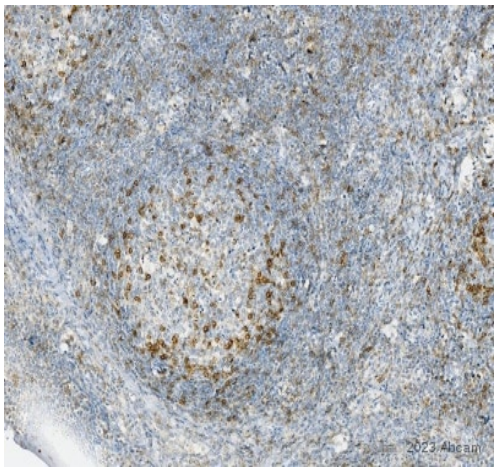


Tissue Microarray (TMA) data for ab237728

Normal tissue samples						Malignant tissue samples					
Human cardiac muscle	x	Human placenta	x			Clear cell carcinoma of human kidney	x	Human glioma	x		
Human cerebrum	x	Human skeletal muscle	x			Human bladder cancer	x	Human hepatocellular carcinoma	x		
Human colon	x (immune cells ✓)	Human skin	x			Human breast carcinoma	x	Human lung carcinoma	x (immune cells ✓)		
Human endometrium	x	Human spleen	x			Human cervical carcinoma	x	Human ovarian carcinoma	x		
Human kidney	x	Human stomach	x (immune cells ✓)			Human colon carcinoma	x (immune cells ✓)	Human pancreatic carcinoma	x		
Human liver	x	Human testis	x			Human endometrial carcinoma	x	Human thyroid carcinoma	x		
Human lung	x	Human thyroid	x			Human gastric adenocarcinoma	x (immune cells ✓)				
Human mammary gland	x	Human tonsil	✓								
Human pancreas	x										

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD1 antibody [CAL20] (ab237728)

Tissue Microarrays stained for "Anti-PD1 antibody [CAL20]" using "ab237728" 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 30 minutes. The sections were incubated with ab237728 for 15 mins at room temperature followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PD1 antibody [CAL20] (ab237728)

This image is courtesy of Dr. Chi Ngai Chan

Immunohistochemical analysis of paraffin-embeded Rhesus monkey tonsil tissue labeling PD1 with **ab251613** followed by Polink 1 Polymer HRP anti-Rabbit IgG.

Heat mediated antigen retrieval-Buffer/Enzyme Used: Dako pH9.

This data was developed using the same antibody clone in a different buffer formulation (**ab251613**).

Anti-PD1 antibody [CAL20] (ab237728)

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