abcam

ab225894

Anti-EpCAM/CD326 antibody [EPR20532-225] - BSA and Azide free

Anti-EpCAM/CD326 antibody [EPR20532-225] - BSA and Azide free (ab225894) is a rabbit recombinant monoclonal antibody in a PBS only buffer for easy conjugation detecting EpCAM/CD326 in **Western Blot, Flow Cytometry, IP, IHC-P, ICC/IF, mIHC**. Suitable for **Human**.

- KO validated for confirmed specificity
- BSA, sodium azide, and glycerol-free for easy conjugation
- Multiplex IHC validated on the Leica BOND® MAX using Opal reagents
- Biophysical QC for unrivalled batch-batch consistency

Recombinant | RabMAb | Advanced Validation | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EPR20532-225
Purification technique	Affinity purification Protein A
Concentration	0.99 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

ICC/IF

Tested

Species	Human
Dilution info	-
Notes	-

IP

Tested

Species	Human
Dilution info	-
Notes	-

Flow Cyt

Tested

Species	Human
Dilution info	-
Notes	-

WB

Tested

Species	Human
Dilution info	-
Notes	-

mIHC

Tested

Species	Human
Dilution info	-
Notes	-

IHC-P

Tested

Species	Human
Dilution info	-
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Target data

See full target information EpCAM/CD326

Function	May act as a physical homophilic interaction molecule between intestinal epithelial cells (IECs) and intraepithelial lymphocytes (IELs) at the mucosal epithelium for providing immunological barrier as a first line of defense against mucosal infection. Plays a role in embryonic stem cells proliferation and differentiation. Up-regulates the expression of FABP5, MYC and cyclins A and E.

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

What is this antibody validated in?
Anti-EpCAM antibody [EPR20532-225] - BSA and Azide free (ab225894) is a rabbit recombinant monoclonal antibody and is validated for use in Western Blot (WB), Flow Cytometry (Flow Cyt), Immunoprecipitation (IP), Immunohistochemistry (IHC-P), Immunocytochemistry/immunofluorescence (ICC/IF), Multiplex IHC (mIHC) in Human samples.

What is the molecular weight of EpCAM?
Anti-EpCAM [EPR20532-225] - BSA and Azide free (ab225894) specifically detects a band for EpCAM (UniProt: P16422) at a molecular weight of 35kDa.

Specificity confirmed

The specificity of Anti-EpCAM antibody [EPR20532-225] - BSA and Azide free (ab225894) has been confirmed by Western blot testing in EPCAM Knockout A431 cell line, ab261902.

Other related products
We have a range of other formats of antibody clone [EPR20532-225] also available for your convenience: ab223582, Carrier free - ab225894, Alexa Fluor® 488 - ab237395, Alexa Fluor® 647 - ab237396, PE - ab237397, APC - ab237398, Alexa Fluor® 594 - ab286811, Biotin - ab314940

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Conjugation ready
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. 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.

Compatibility
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.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	EpCAM also known as epithelial cell adhesion molecule is a transmembrane glycoprotein with a molecular weight of around 40 kDa. Alternative names include CD326 and TACSTD1. This protein is expressed on the surface of most epithelial cells and plays a role in cell adhesion. It is involved in maintaining the integrity of epithelial tissues by mediating homotypic cell-cell interactions which are important for the structure and function of tissues.
Biological function summary	EpCAM facilitates signaling pathways that impact cellular processes like proliferation and differentiation. It is not a part of a larger protein complex but interacts directly with other cellular machinery to transmit intracellular signals. EpCAM influences stem cell behavior and is important in tissue homeostasis assisting in both normal regenerative processes and wound healing.

Pathways	EpCAM participates in regulatory mechanisms involving the Wnt/B-catenin signaling pathway which affects gene expression and cell fate decisions. Through its action EpCAM modulates components like E-cadherin which is important for cell adhesion and the maintenance of the epithelial layer. It indirectly influences pathways relating to MYC a transcription factor that regulates cell cycle progression and apoptosis.
Associated diseases and disorders	EpCAM is associated with various types of cancer including colorectal and breast cancer. Its overexpression often correlates with aggressive cancer phenotypes and poor prognosis. Besides cancers EpCAM mutations can lead to congenital tufting enteropathy a rare condition affecting intestinal epithelial structure and function. Aberrant EpCAM expression affects B-catenin levels impacting cellular pathways linked to tumor progression and epithelial integrity.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

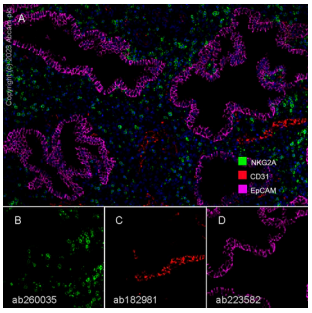
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

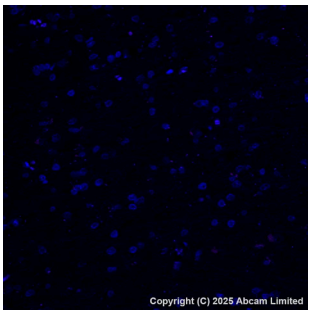
24 product images



Multiplex immunohistochemistry - Anti-EpCAM/CD326 antibody [EPR20532-225] - BSA and Azide free (ab225894)

EpCAM/CD326 Multiplex immunohistochemistry staining of Human endometrium tissue using rabbit Anti-EpCAM/CD326 antibody

Fluorescence multiplex immunohistochemical analysis of the human endometrium (Formalin/PFA-fixed paraffin-embedded sections). Panel A: merged staining of anti-EpCAM (ab223582, magenta; Opal™690), anti-NKG2A (ab260035, green; Opal™520) and anti-CD31 (ab182981, red; Opal™570) on human endometrium. Panel B: anti-NKG2A stained on NK cells. Panel C: anti-CD31 stained on endothelial cells. Panel D: anti-EpCAM stained on glandular cells. Opal Polymer HRP Ms + Rb was used as a secondary antibody. The section was incubated in three rounds of staining: in the order of ab223582 at 1/500 dilution (1.008 µg/ml), ab260035 at 1/2000 dilution (0.262 µg/ml) and ab182981 at 1/4000 dilution (0.137 µg/ml) 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. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins. DAPI (blue) was used as a nuclear counter stain. This data was developed using the same antibody clone in a different buffer formulation (ab223582).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-EpCAM/CD326 antibody [EPR20532-225] - BSA and Azide free (ab225894)

EpCAM/CD326 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human cerebrum tissue using rabbit Anti-EpCAM/CD326 antibody

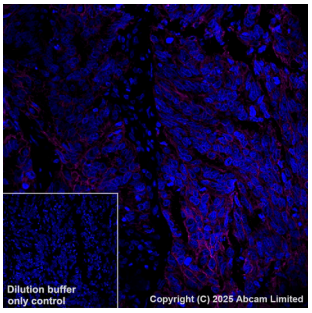
This data was developed using ab237396, the same antibody clone in a different buffer formulation.
Immunohistochemical analysis of paraffin-embedded human cerebrum tissue labeling EpCAM with ab237396 at 1/50 (10 µg/ml) dilution.

Negative control: no staining on human cerebrum.

The primary antibody was incubated overnight at 4°C (shown in magenta). Nuclear DNA was labelled with DAPI (shown in blue). The section was then mounted using Fluoromount®.

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

Heat mediated antigen retrieval was performed using ab93684 (Tris/EDTA buffer, pH 9.0).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-EpCAM/CD326 antibody [EPR20532-225] - BSA and Azide free (ab225894)

EpCAM/CD326 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human colon cancer tissue using rabbit Anti-EpCAM/CD326 antibody

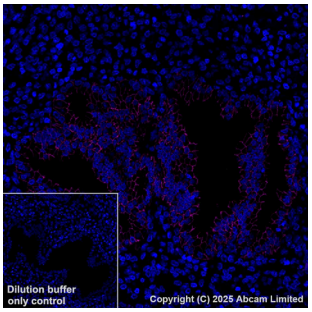
This data was developed using ab237396, the same antibody clone in a different buffer formulation.
Immunohistochemical analysis of paraffin-embedded human colon cancer tissue labeling EpCAM with ab237396 at 1/50 (10 µg/ml) dilution.

Membrane staining on human colon cancer.

The primary antibody was incubated overnight at 4°C (shown in magenta). Nuclear DNA was labelled with DAPI (shown in blue). The section was then mounted using Fluoromount®.

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

Heat mediated antigen retrieval was performed using ab93684 (Tris/EDTA buffer, pH 9.0).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-EpCAM/CD326 antibody [EPR20532-225] - BSA and Azide free (ab225894)

EpCAM/CD326 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human endometrium tissue using rabbit Anti-EpCAM/CD326 antibody

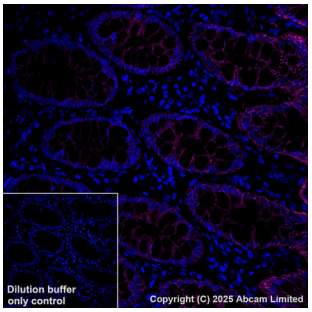
This data was developed using ab237396, the same antibody clone in a different buffer formulation.
Immunohistochemical analysis of paraffin-embedded human endometrium tissue labeling EpCAM with ab237396 at 1/50 (10 µg/ml) dilution.

Membrane staining on human endometrium.

The primary antibody was incubated overnight at 4°C (shown in magenta). Nuclear DNA was labelled with DAPI (shown in blue). The section was then mounted using Fluoromount®.

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

Heat mediated antigen retrieval was performed using ab93684 (Tris/EDTA buffer, pH 9.0).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-EpCAM/CD326 antibody [EPR20532-225] - BSA and Azide free (ab225894)

EpCAM/CD326 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human colon tissue using rabbit Anti-EpCAM/CD326 antibody

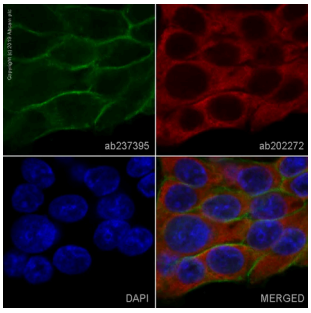
This data was developed using ab237396, the same antibody clone in a different buffer formulation.
Immunohistochemical analysis of paraffin-embedded human colon tissue labeling EpCAM with ab237396 at 1/50 (10 µg/ml) dilution.

Membrane staining on human colon.

The primary antibody was incubated overnight at 4°C (shown in magenta). Nuclear DNA was labelled with DAPI (shown in blue). The section was then mounted using Fluoromount®.

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

Heat mediated antigen retrieval was performed using ab93684 (Tris/EDTA buffer, pH 9.0).

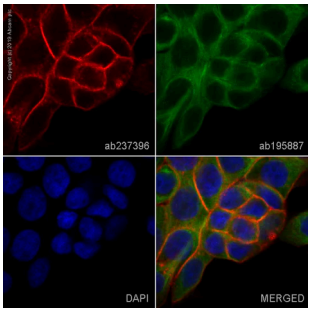


Immunocytochemistry/ Immunofluorescence - Anti-EpCAM/CD326 antibody [EPR20532-225] - BSA and Azide free (ab225894)

EpCAM/CD326 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-EpCAM/CD326 antibody

Clone EPR20532-225 (ab225894) has been successfully conjugated by Abcam. This image was generated using Anti-EpCAM antibody [EPR20532-225] (Alexa Fluor® 488). Please refer to ab237395 for protocol details.
ab237395 staining EpCAM in HT-29 cells. The cells were fixed with 100% methanol (5 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 ab237395 at 1/1000 dilution (shown in green) and ab202272, Rabbit monoclonal to alpha Tubulin (Alexa Fluor® 594), at 1/250 dilution (shown in red). Nuclear DNA was labeled with DAPI (shown in blue).

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



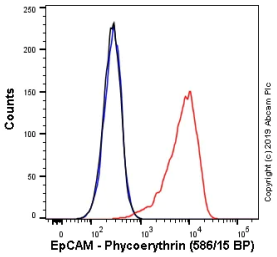
Immunocytochemistry/ Immunofluorescence - Anti-EpCAM/CD326 antibody [EPR20532-225] - BSA and Azide free (ab225894)

EpCAM/CD326 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-EpCAM/CD326 antibody

Clone EPR20532-225 (ab225894) has been successfully conjugated by Abcam. This image was generated using Anti-EpCAM antibody [EPR20532-225] (Alexa Fluor® 647). Please refer to ab237396 for protocol details.
ab237396 staining EpCAM in HT-29 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 ab237396 at 1/100 dilution (shown in red) and ab195887, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 488), at 1/250 dilution (shown in). Nuclear DNA was labeled with DAPI (shown in blue).

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

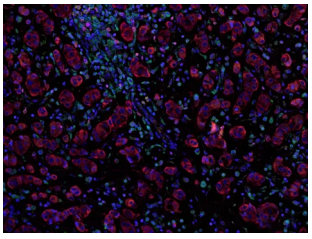
This product also gave a positive signal under the same testing conditions in HT-29 cells fixed with 100% methanol (5 min).



Flow Cytometry - Anti-EpCAM/CD326 antibody [EPR20532-225] - BSA and Azide free (ab225894)

EpCAM/CD326 Flow Cytometry staining using rabbit Anti-EpCAM/CD326 antibody

Clone EPR20532-225 (ab225894) has been successfully conjugated by Abcam. This image was generated using Anti-EpCAM antibody [EPR20532-225] (PE). Please refer to ab237397 for protocol details.
Overlay histogram showing HCT116 cells stained with ab237397 (red line). The cells were then incubated in 1x PBS / 10% normal Goat serum to block non-specific protein-protein interactions followed by the antibody (ab237397, 1/6250 dilution) for 30 min at 4°C. Isotype control antibody (black line) was Rabbit IgG (monoclonal) Phycoerythrin (ab209478) used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 50 mW Yellow/Green laser (561nm) and 586/15 bandpass filter.



This image is courtesy of ImmunoAtlas.

Multiplex immunohistochemistry - Anti-EpCAM/CD326 antibody [EPR20532-225] - BSA and Azide free (ab225894)

EpCAM/CD326 Multiplex immunohistochemistry staining using rabbit Anti-EpCAM/CD326 antibody

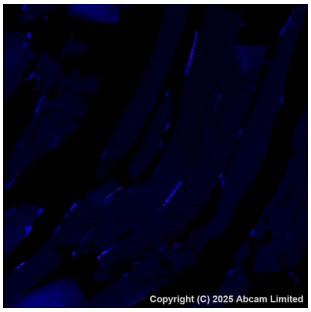
Fluorescence multiplex immunohistochemical analysis of Human breast cancer tissue (formalin-fixed paraffin-embedded section).
Merged staining of Anti-PD-L1 (ab251611; cyan; Opal™ 520), Anti-Granzyme B (ab219803; yellow; Opal™ 540), Anti-PD1 (ab251613; magenta; Opal™ 570), Anti-pan Cytokeratin (ab264485; 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 was developed using the same antibody clone in a different buffer formulation (ab223582).

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



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-EpCAM/CD326 antibody [EPR20532-225] - BSA and Azide free (ab225894)

EpCAM/CD326 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human skeletal muscle tissue using rabbit Anti-EpCAM/CD326 antibody

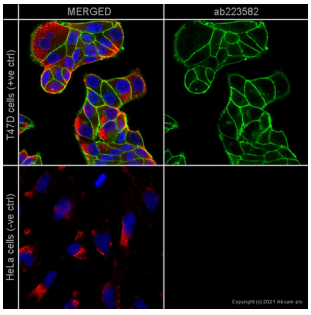
This data was developed using ab237396, the same antibody clone in a different buffer formulation.
Immunohistochemical analysis of paraffin-embedded human skeletal muscle tissue labeling EpCAM with ab237396 at 1/50 (10 µg/ml) dilution.

Negative control: no staining on human skeletal muscle.

The primary antibody was incubated overnight at 4°C (shown in magenta). Nuclear DNA was labelled with DAPI (shown in blue). The section was then mounted using Fluoromount®.

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

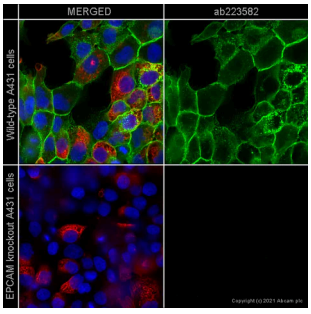
Heat mediated antigen retrieval was performed using ab93684 (Tris/EDTA buffer, pH 9.0).



Immunocytochemistry/ Immunofluorescence - Anti-EpCAM/CD326 antibody [EPR20532-225] - BSA and Azide free (ab225894)

EpCAM/CD326 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-EpCAM/CD326 antibody

This data was developed using the same antibody clone in a different buffer formulation (ab223582). ab223582 staining EpCAM in T47D positive cells (top panel) and HeLa negative cells (bottom panel). The cells were fixed with 4% paraformaldehyde (10 min) then permeabilized with 0.1% PBS-Tween 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 with ab223582 at 0.1µg/ml concentration and ab7291 (Mouse monoclonal to alpha Tubulin) at 1/1000 dilution overnight at 4°C followed by a further incubation at room temperature for 1h with a goat secondary antibody to rabbit IgG (Alexa Fluor® 488) (ab150081) at 2 µg/ml (shown in green) and a goat secondary antibody to mouse IgG (Alexa Fluor® 594) (ab150120) at 2 µg/ml (shown in red). Nuclear DNA was labelled in blue with DAPI. This antibody performed similarly using 100% methanol fixation. Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a single confocal section is shown.

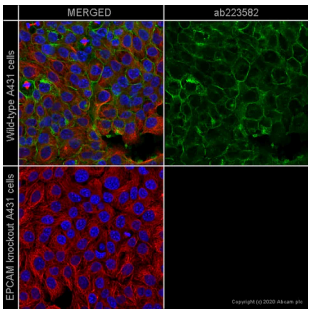


Immunocytochemistry/ Immunofluorescence - Anti-EpCAM/CD326 antibody [EPR20532-225] - BSA and Azide free (ab225894)

EpCAM/CD326 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-EpCAM/CD326 antibody

This data was developed using the same antibody clone in a different buffer formulation (ab223582). ab223582 staining EpCAM in wild-type A431 cells (top panel) and EpCAM knockout A431 cells (bottom panel). The cells were fixed with 4% paraformaldehyde (10 min) then permeabilized with 0.1% PBS-Tween 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 with ab223582 at 0.1µg/ml concentration and ab7291 (Mouse monoclonal to alpha Tubulin) at 1/1000 dilution overnight at 4°C followed by a further incubation at room temperature for 1h with a goat secondary antibody to rabbit IgG (Alexa Fluor® 488) (ab150081) at 2 µg/ml (shown in green) and a goat secondary antibody to mouse IgG (Alexa Fluor® 594) (ab150120) at 2 µg/ml (shown in red).

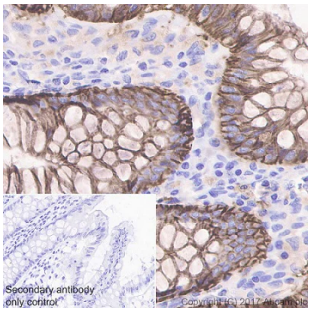
Nuclear DNA was labelled in blue with DAPI. This antibody performed similarly using 100% methanol fixation. Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a single confocal section is shown.



Immunocytochemistry/ Immunofluorescence - Anti-EpCAM/CD326 antibody [EPR20532-225] - BSA and Azide free (ab225894)

EpCAM/CD326 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-EpCAM/CD326 antibody

This data was developed using the same antibody clone in a different buffer formulation (ab223582). ab223582 staining EpCAM in wild-type A431 cells (top panel) and EpCAM knockout A431 cells (bottom panel). The cells were fixed with 100% methanol (5 min) then permeabilized with 0.1% PBS-Tween 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 with ab223582 at 1/5000 dilution and ab7291 (Mouse monoclonal to alpha Tubulin) at 1/1000 dilution overnight at 4°C followed by a further incubation at room temperature for 1h with a goat secondary antibody to rabbit IgG (Alexa Fluor® 488) (ab150081) at 2 µg/ml (shown in green) and a goat secondary antibody to mouse IgG (Alexa Fluor® 594) (ab150120) at 2 µg/ml (shown in red). Nuclear DNA was labelled in blue with DAPI.
Image was taken with a confocal microscope (Leica-Microsystems TCS SP8).



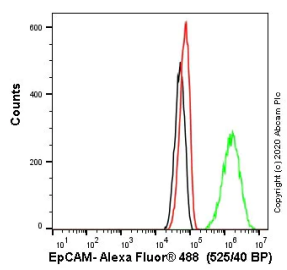
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-EpCAM/CD326 antibody [EPR20532-225] - BSA and Azide free (ab225894)

EpCAM/CD326 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-EpCAM/CD326 antibody

Immunohistochemical analysis of paraffin-embedded human colon tissue labeling EpCAM with ab223582 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Membranous staining on human colon is observed (PMID: 15637741). 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab223582).

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



Flow Cytometry - Anti-EpCAM/CD326 antibody [EPR20532-225] - BSA and Azide free (ab225894)

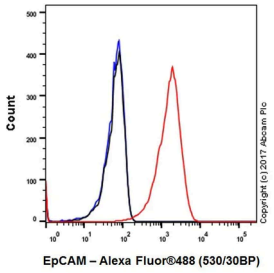
EpCAM/CD326 Flow Cytometry staining using rabbit Anti-EpCAM/CD326 antibody

This data was developed using the same antibody clone in a different buffer formulation (ab225894)
Flow cytometry overlay histogram showing wild-type A431 (green line) and EPCAM knockout A431 cells stained with ab223582 (red line). The cells were incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab223582) ($1x10^6$ in 100µl at 0.2 µg/ml) for 30 min at 4°C.

The secondary antibody Goat anti-rabbit IgG H&L (Alexa Fluor® 488, pre-adsorbed) (ab150081) was used at 1/2000 for 30 min at 4°C.

Isotype control antibody was Rabbit IgG (monoclonal) (ab172730) used at the same concentration and conditions as the primary antibody (wild-type A431 - black line; EPCAM knockout A431 - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity).

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.

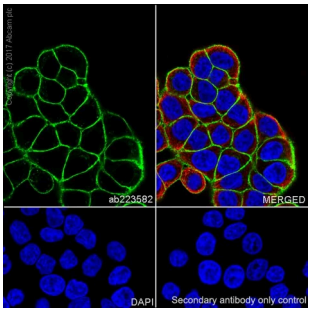


Flow Cytometry - Anti-EpCAM/CD326 antibody [EPR20532-225] - BSA and Azide free (ab225894)

EpCAM/CD326 Flow Cytometry staining using rabbit Anti-EpCAM/CD326 antibody

Flow cytometric analysis of HCT 116 (human colorectal carcinoma cell line) cell line labeling EpCAM with ab223582 at 1/500 (red) compared with a rabbit monoclonal IgG Isotype control (ab172730) (black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat Anti-Rabbit IgG (Alexa Fluor® 488) (ab150077), at 1/2000 dilution was used as the secondary antibody.
Total viable cells were gated for the final data.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab223582).



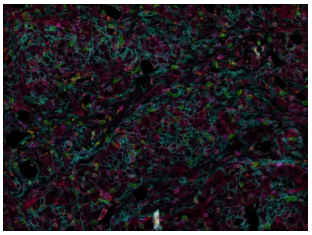
Immunocytochemistry/ Immunofluorescence - Anti-EpCAM/CD326 antibody [EPR20532-225] - BSA and Azide free (ab225894)

EpCAM/CD326 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-EpCAM/CD326 antibody

Immunofluorescent analysis of 4% paraformaledehyde-fixed, 0.1% Triton X-100 permeabilized HT-29 (human colorectal adenocarcinoma cell line) cells labeling EpCAM with ab223582 at 1/500 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 on HT-29 cells.
The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (ab195889) (red) at 1/200 dilution.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is 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 (ab223582).



This image is courtesy of ImmunoAtlas.

Multiplex immunohistochemistry - Anti-EpCAM/CD326 antibody [EPR20532-225] - BSA and Azide free (ab225894)

EpCAM/CD326 Multiplex immunohistochemistry staining using rabbit Anti-EpCAM/CD326 antibody

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

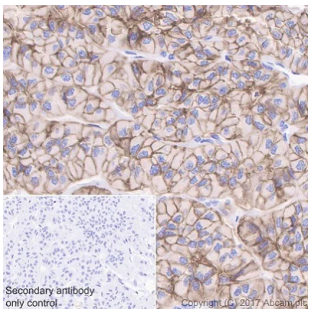
Merged staining of Anti-PD-L1 (ab251611; cyan; Opal™ 520), Anti-Granzyme B (ab219803; yellow; Opal™ 540), Anti-PD1 (ab251613; magenta; Opal™ 570), Anti-pan Cytokeratin (ab264485; red; Opal™ 620), Anti-EpCAM (ab225894; red; Opal™ 620), Anti-CD8 alpha (ab251596; green; Opal™ 650) and Anti-FOXP3 (ab96048; 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 was developed using the same antibody clone in a different buffer formulation (ab223582).

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



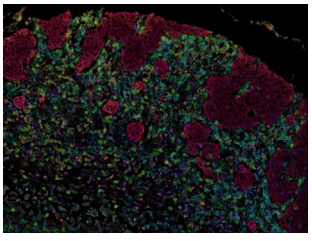
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-EpCAM/CD326 antibody [EPR20532-225] - BSA and Azide free (ab225894)

EpCAM/CD326 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-EpCAM/CD326 antibody

Immunohistochemical analysis of paraffin-embedded human thyroid cancer tissue labeling EpCAM with ab223582 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Membranous staining on human thyroid cancer is observed (PMID: 15637741). 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab223582).

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



This image is courtesy of ImmunoAtlas.

Multiplex immunohistochemistry - Anti-EpCAM/CD326 antibody [EPR20532-225] - BSA and Azide free (ab225894)

EpCAM/CD326 Multiplex immunohistochemistry staining using rabbit Anti-EpCAM/CD326 antibody

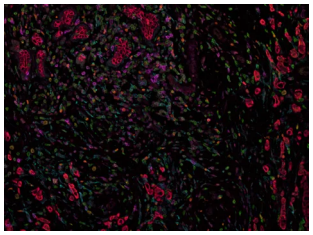
Fluorescence multiplex immunohistochemical analysis of Human breast cancer tissue (formalin-fixed paraffin-embedded section).
Merged staining of Anti-PD-L1 (ab251611; cyan; Opal™ 520), Anti-Granzyme B (ab219803; yellow; Opal™ 540), Anti-PD1 (ab251613; magenta; Opal™ 570), Anti-pan Cytokeratin (ab264485; 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 was developed using the same antibody clone in a different buffer formulation (ab223582).

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



This image is courtesy of ImmunoAtlas.

Multiplex immunohistochemistry - Anti-EpCAM/CD326 antibody [EPR20532-225] - BSA and Azide free (ab225894)

EpCAM/CD326 Multiplex immunohistochemistry staining using rabbit Anti-EpCAM/CD326 antibody

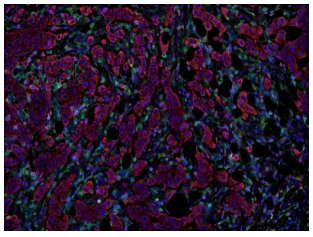
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Merged staining of Anti-PD-L1 (ab251611; cyan; Opal™ 520), Anti-Granzyme B (ab219803; yellow; Opal™ 540), Anti-PD1 (ab251613; magenta; Opal™ 570), Anti-pan Cytokeratin (ab264485; red; Opal™ 620), Anti-EpCAM (ab225894; red; Opal™ 620), Anti-CD8 alpha (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.

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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.

This data was developed using the same antibody clone in a different buffer formulation (ab223582).



Multiplex immunohistochemistry - Anti-EpCAM/CD326 antibody [EPR20532-225] - BSA and Azide free (ab225894)

EpCAM/CD326 Multiplex immunohistochemistry staining using rabbit Anti-EpCAM/CD326 antibody

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

This image is courtesy of ImmunoAtlas.

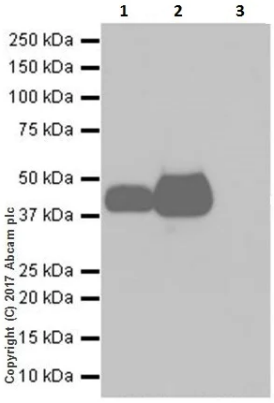
Merged staining of Anti-PD-L1 (ab251611; cyan; Opal™ 520), Anti-Granzyme B (ab219803; yellow; Opal™ 540), Anti-PD1 (ab251613; magenta; Opal™ 570), Anti-pan Cytokeratin (ab264485; red; Opal™ 620), Anti-EpCAM (ab225894; red; Opal™ 620), Anti-CD8 alpha (ab251596; green; Opal™ 650) and Anti-FOXP3 (ab96048;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

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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.

This data was developed using the same antibody clone in a different buffer formulation (ab223582).



Immunoprecipitation - Anti-EpCAM/CD326 antibody [EPR20532-225] - BSA and Azide free (ab225894)

EpCAM was immunoprecipitated from 0.35 mg of HCT 116 (human colorectal carcinoma cell line) whole cell lysate with ab223582 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab223582 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/10000 dilution.
Lane 1: HCT 116 lysate 10 µg (Input).

Lane 2: ab223582 IP in HCT 116 lysate.

Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab223582 in HCT 116 whole cell lysate.

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

Exposure time: 30 seconds.

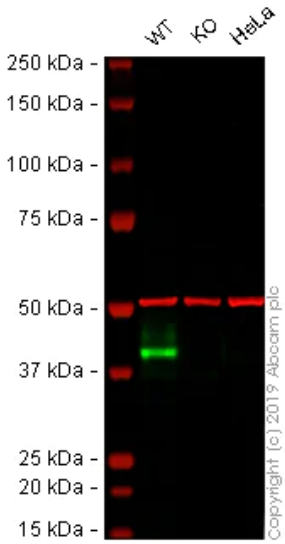
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab223582).

All lanes:
Immunoprecipitation - Anti-EpCAM/CD326 antibody [EPR20532-225] (ab223582)

Predicted band size: 35 kDa

Observed band size: 42 kDa

Exposure time: 30s



Western blot - Anti-EpCAM/CD326 antibody [EPR20532-225] - BSA and Azide free (ab225894)

EpCAM/CD326 Western blot staining using rabbit Anti-EpCAM/CD326 antibody

This data was developed using the same antibody clone in a different buffer formulation (ab223582).
Lanes 1 - 3: Merged signal (red and green). Green - ab223582 observed at 40 kDa. Red - loading control, ab7291 (Mouse anti-Alpha Tubulin [DM1A] observed at 55kDa.

ab223582 was shown to react with EpCAM in A431 wild-type cells in Western blot. Loss of signal was observed when EpCAM knockout sample was used. A431 wild-type and EpCAM knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% Milk in TBS-T (0.1% Tween®) before incubation with ab223582 and ab7291 (Mouse anti-Alpha Tubulin [DM1A] overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

All lanes:
Western blot - Anti-EpCAM/CD326 antibody [EPR20532-225] (ab223582) at 1/1000 dilution

Lane 1:
Wild-type A-431 (Human epidermoid carcinoma cell line) whole cell lysate at 20 µg

Lane 2:
Western blot - Human EPCAM knockout A-431 cell lysate (ab261708) at 20 µg

Lane 3:
HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate at 20 µg

Performed under reducing conditions.

Predicted band size: 35 kDa

Observed band size: 40 kDa

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.