abcam

Product datasheet

Anti-EpCAM antibody [EPR20533-266] ab213501

Recombinant RabMAb

8 Images

Overview

Product name Anti-EpCAM antibody [EPR20533-266]

Description Rabbit monoclonal [EPR20533-266] to EpCAM

Host species Rabbit

Suitable for: WB, ICC/IF, IP, IHC-P **Tested applications**

Species reactivity Reacts with: Mouse, Rat

Immunogen Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: Mouse small intestine, pancreas, colon and kidney lysates; Rat colon and kidney lysates; 4T1

whole cell lysate. IHC-P: Mouse colon and lung tissues; Rat colon tissue. ICC/IF: 4T1 cells. IP: 4T1

whole cell lysate.

General notes This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility

- Improved sensitivity and specificity

- Long-term security of supply

- Animal-free production

For more information see here.

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

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: 0.01% Sodium azide

Constituents: PBS, 0.05% BSA, 40% Glycerol (glycerin, glycerine)

Purity Protein A purified

Clonality Monoclonal Clone number

EPR20533-266

Isotype IgG

Applications

The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab213501 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
WB		1/1000. Detects a band of approximately 39 kDa (predicted molecular weight: 35 kDa).
ICC/IF		1/100.
IP		1/40.
IHC-P		1/2000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Target

Function

Tissue specificity

Involvement in disease

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.

Highly and selectively expressed by undifferentiated rather than differentiated embryonic stem cells (ESC). Levels rapidly diminish as soon as ESC's differentiate (at protein levels). Expressed in almost all epithelial cell membranes but not on mesodermal or neural cell membranes. Found on the surface of adenocarcinoma.

Defects in EPCAM are the cause of diarrhea type 5 (DIAR5) [MIM:613217]. It is an intractable diarrhea of infancy characterized by villous atrophy and absence of inflammation, with intestinal epithelial cell dysplasia manifesting as focal epithelial tufts in the duodenum and jejunum. Defects in EPCAM are a cause of hereditary non-polyposis colorectal cancer type 8 (HNPCC8) [MIM:613244]. HNPCC is a disease associated with marked increase in cancer susceptibility. It is characterized by a familial predisposition to early-onset colorectal carcinoma (CRC) and extracolonic tumors of the gastrointestinal, urological and female reproductive tracts. HNPCC is reported to be the most common form of inherited colorectal cancer in the Western world. Clinically, HNPCC is often divided into two subgroups. Type I is characterized by hereditary predisposition to colorectal cancer, a young age of onset, and carcinoma observed in the proximal colon. Type II is characterized by increased risk for cancers in certain tissues such as the uterus, ovary, breast, stomach, small intestine, skin, and larynx in addition to the colon. Diagnosis of classical HNPCC is based on the Amsterdam criteria: 3 or more relatives affected by colorectal cancer, one a first degree relative of the other two; 2 or more generation affected; 1 or more colorectal cancers presenting before 50 years of age; exclusion of hereditary polyposis syndromes. The term 'suspected HNPCC' or 'incomplete HNPCC' can be used to describe families who do not or only partially fulfill the Amsterdam criteria, but in whom a genetic basis for colon cancer is strongly suspected. Note=HNPCC8 results from heterozygous deletion of 3-prime exons of EPCAM and intergenic regions directly upstream of MSH2, resulting in transcriptional read-through and epigenetic silencing of MSH2 in tissues expressing EPCAM.

Sequence similarities Belongs to the EPCAM family.

Contains 1 thyroglobulin type-1 domain.

Post-translational modifications

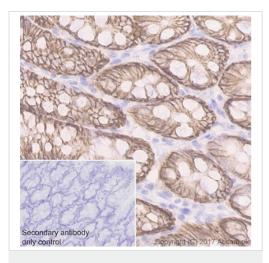
Hyperglycosylated in carcinoma tissue as compared with autologous normal epithelia.

Glycosylation at Asn-198 is crucial for protein stability.

Cellular localization Lateral cell membrane. Cell junction > tight junction. Co-localizes with CLDN7 at the lateral cell

membrane and tight junction.

Images



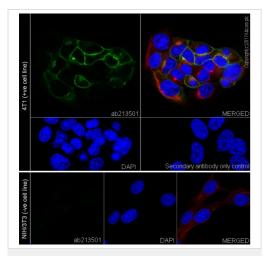
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-EpCAM antibody

[EPR20533-266] (ab213501)

Immunohistochemical analysis of paraffin-embedded rat colon tissue labeling EpCAM with ab213501 at 1/2000 dilution, followed by Goat anti-Rabbit lgG H&L (HRP) Ready to use. Membranous staining on rat 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 lgG H&L (HRP) Ready to use.

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



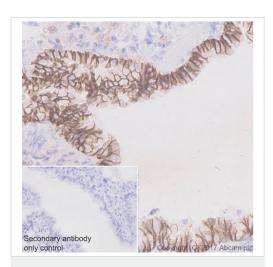
Immunocytochemistry/ Immunofluorescence - Anti-EpCAM antibody [EPR20533-266] (ab213501)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized 4T1 (mouse mammary gland carcinoma cell line) and NIH/3T3 (mouse embyro fibroblast cell line) cells labeling EpCAM with ab213501 at 1/100 dilution followed by Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing membranous staining on 4T1 cells.

Negative control: NIH/3T3 (PMID:23264216).

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 lgG H&L (Alexa Fluor[®] 488) (ab150077) secondary antibody at 1/1000 dilution.



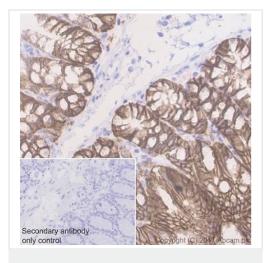
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-EpCAM antibody

[EPR20533-266] (ab213501)

Immunohistochemical analysis of paraffin-embedded mouse lung tissue labeling EpCAM with ab213501 at 1/2000 dilution, followed by Goat anti-Rabbit lgG H&L (HRP) Ready to use. Membranous staining on mouse lung is observed (PMID: 15637741). Counter stained with hematoxylin.

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

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

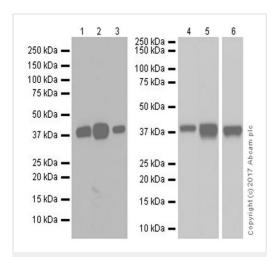


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-EpCAM antibody
[EPR20533-266] (ab213501)

Immunohistochemical analysis of paraffin-embedded mouse colon tissue labeling EpCAM with ab213501 at 1/2000 dilution, followed by Goat anti-Rabbit lgG H&L (HRP) Ready to use. Membranous staining on mouse 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 lgG H&L (HRP) Ready to use.

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



Western blot - Anti-EpCAM antibody [EPR20533-266] (ab213501)

Lanes 1-3: Anti-EpCAM antibody [EPR20533-266] (ab213501) at 1/1000 dilution

Lanes 4-6: Anti-EpCAM antibody [EPR20533-266] (ab213501) at 1/5000 dilution

Lane 1: Mouse small intestine lysate

Lane 2: Mouse pancreas lysate

Lane 3: Rat colon lysate

Lane 4: Mouse colon lysate

Lane 5: Mouse kidney lysate

Lane 6: Rat kidney lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

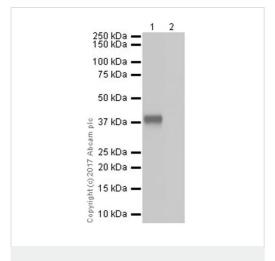
Developed using the ECL technique.

Predicted band size: 35 kDa
Observed band size: 39 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1-3: 3 minutes; Lane 4/5: 5 seconds; Lane 6: 30 seconds.

The MW observed is consistent with the literature (PMID 23409978; PMID 23618806).



Western blot - Anti-EpCAM antibody [EPR20533-266] (ab213501) **All lanes :** Anti-EpCAM antibody [EPR20533-266] (ab213501) at 1/5000 dilution

Lane 1 : 4T1 (mouse mammary gland carcinoma cell line) whole cell lysate

Lane 2: NIH/3T3 (mouse embyro fibroblast cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit $\lg G \ H\&L \ (HRP) \ (\underline{ab97051})$ at 1/100000 dilution

Developed using the ECL technique.

Predicted band size: 35 kDa Observed band size: 39 kDa

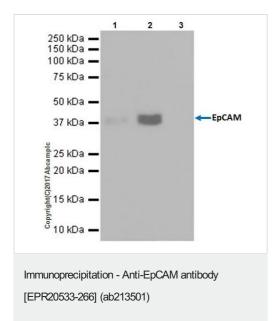
Exposure time: 5 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

The MW observed is consistent with the literature (PMID 23409978; PMID 23618806).

Negative control:

NIH/3T3 (PMID:23264216).



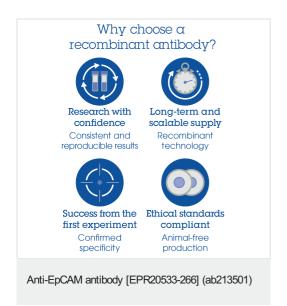
EpCAM was immunoprecipitated from 0.35 mg of 4T1 (mouse mammary gland carcinoma cell line) whole cell lysate with ab213501 at 1/40 dilution. Western blot was performed from the immunoprecipitate using ab213501 at 1/1000 dilution. VeriBlot for IP secondary antibody (HRP) (ab13136), was used as secondary antibody at 1/10000 dilution.

Lane 1: 4T1 whole cell lysate 10 µg (Input).

Lane 2: ab213501 IP in 4T1 whole cell lysate.

Lane 3: Rabbit monoclonal IgG (<u>ab172730</u>) instead of ab213501 in 4T1 whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST. Exposure time: 3 minutes.



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