abcam

Product datasheet

Anti-EpCAM antibody [EPR20532-222] ab213500

敲除验证 重组 RabMAb

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概述

产品名称	Anti-EpCAM抗体[EPR20532-222]
描述	兔单克隆抗体[EPR20532-222] to EpCAM
宿主	Rabbit
经测试应用	**适用于:** IHC-P, WB, IP, ICC/IF
种属反应性	**与反应:** Mouse, Rat, Human
免疫原	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
阳性对照	WB: A431, T-47D, SK-OV-3, HT-29, HCT 116, MCF7 and 4T1 whole cell lysates; Human breast, colon and fetal kidney lysates; Mouse small intestine lysate. IHC-P: Human colon, breast carcinoma and endometrium cancer tissues; Mouse and rat colon tissues. ICC/IF: HCT 116 and 4T1 cells. IP: HCT 116 whole cell lysate.
常规说明	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 short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
存储溶液	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
纯度	Protein A purified
克隆	单克隆
克隆编号	EPR20532-222

同种型 IgG

应用

The Abpromise guarantee Abpromise™承诺保证使用ab213500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
IHC-P		1/16000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB	★★☆☆☆ (1)	1/1000. Detects a band of approximately 36, 40 kDa (predicted molecular weight: 35 kDa).
IP		1/40.
ICC/IF		1/100.

靶标

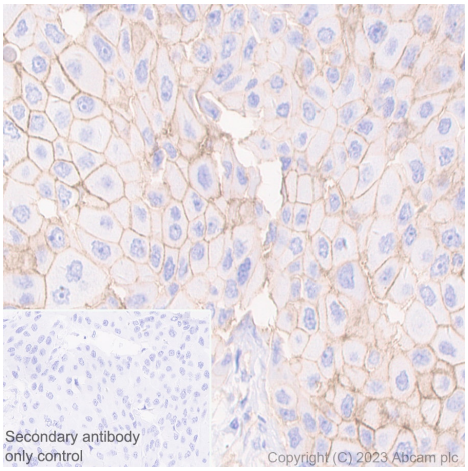
功能 May act as a physical homophilic interaction molecule between intestinal epithelial cells (IECs) and intraepithelial lymphocytes (IELs) at the mucosal epithelium for providing immunological barrier as a first line of defense against mucosal infection. Plays a role in embryonic stem cells proliferation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

组织特异性 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.

疾病相关 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 extra-colonic 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

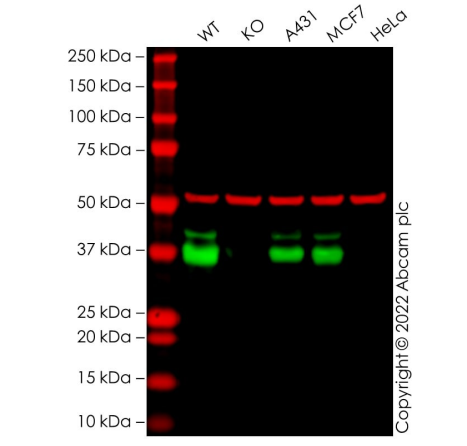
	read-through and epigenetic silencing of MSH2 in tissues expressing EPCAM.
序列相似性	Belongs to the EPCAM family. Contains 1 thyroglobulin type-1 domain.
翻译后修饰	Hyperglycosylated in carcinoma tissue as compared with autologous normal epithelia. Glycosylation at Asn-198 is crucial for protein stability.
细胞定位	Lateral cell membrane. Cell junction > tight junction. Co-localizes with CLDN7 at the lateral cell membrane and tight junction.

图片



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-EpCAM antibody [EPR20532-222] (ab213500)

Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue labeling EpCAM with ab213500 at 1/16000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab214880**) Ready to use.
Counter stained with Hematoxylin.
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (**ab214880**).
Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
Positive staining on human breast carcinoma. The section was incubated with ab213500 at 4°C overnight.



Western blot - Anti-EpCAM antibody [EPR20532-222] (ab213500)

All lanes : Anti-EpCAM antibody [EPR20532-222] (ab213500) at 1/1000 dilution

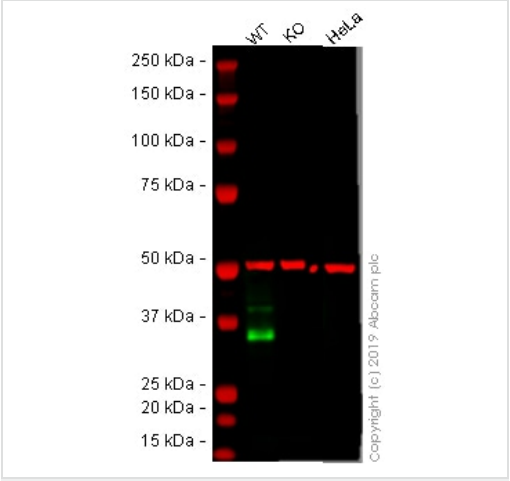
Lane 1 : Wild-type HCT 116 cell lysate
Lane 2 : EPCAM knockout HCT 116 cell lysate
Lane 3 : A431 cell lysate
Lane 4 : MCF7 cell lysate
Lane 5 : HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 35 kDa
Observed band size: 37-45 kDa

False colour image of Western blot: Anti-EpCAM antibody [EPR20532-222] staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab213500 was shown to bind specifically to EpCAM. A band was observed at 37/45 kDa in wild-type HCT 116 cell lysates with no signal observed at this size in EPCAM knockout cell line **ab281596** (knockout cell lysate **ab282948**). To generate this image, wild-type and EPCAM knockout HCT 116 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 5 % milk in TBS-0.1 % Tween® 20 (TBS-T) 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 (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) at 1/20000 dilution.



Western blot - Anti-EpCAM antibody [EPR20532-222] (ab213500)

All lanes : Anti-EpCAM antibody [EPR20532-222] (ab213500) at 1/1000 dilution

Lane 1 : Wild-type A431 whole cell lysate
Lane 2 : EPCAM knockout A431 whole cell lysate
Lane 3 : HeLa whole cell lysate

Lysates/proteins at 20 µg per lane.

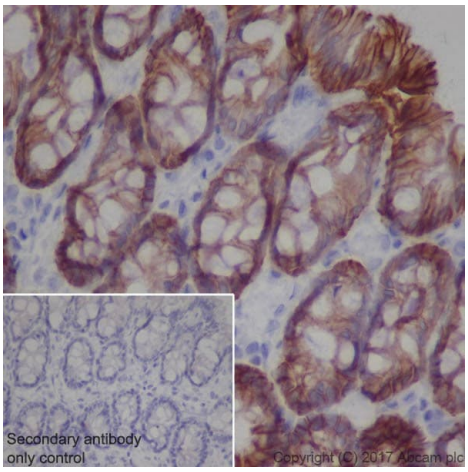
Performed under reducing conditions.

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

Lanes 1 - 3: Merged signal (red and green). Green - ab213500 observed at 40 kDa. Red - loading control, **ab7291** (Mouse anti-Alpha Tubulin [DM1A] observed at 55kDa.

ab213500 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 ab213500 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.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-EpCAM antibody [EPR20532-222] (ab213500)

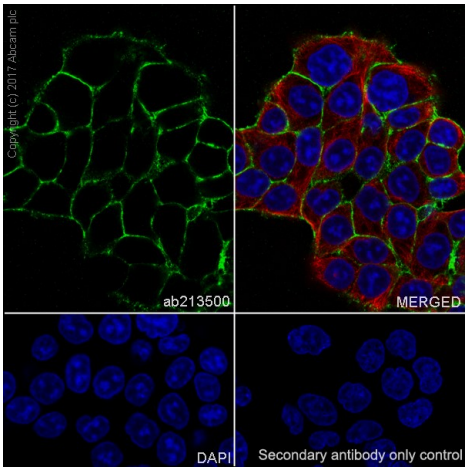
Immunohistochemical analysis of paraffin-embedded rat colon tissue labeling EpCAM with ab213500 at 1/16000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

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 IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

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



Immunocytochemistry/ Immunofluorescence - Anti-EpCAM antibody [EPR20532-222] (ab213500)

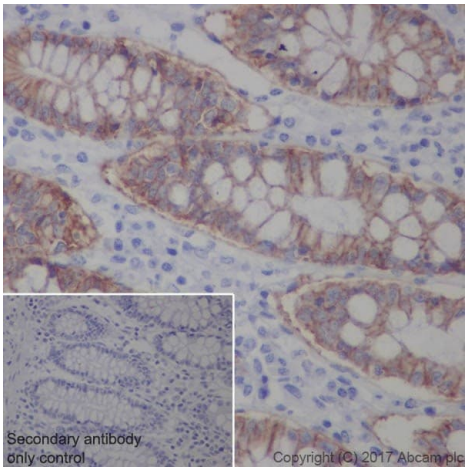
Immunofluorescent analysis of 100% methanol-fixed HCT 116 (Human colorectal carcinoma cell line) cells labeling EpCAM with ab213500 at 1/100 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green).

Confocal image showing membranous staining on HCT 116 cells.

The nuclear counterstain is DAPI (blue).

Tubulin is detected with **ab195889** (Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594)) at 1/200 dilution (red).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) at 1/1000 dilution.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-EpCAM antibody [EPR20532-222] (ab213500)

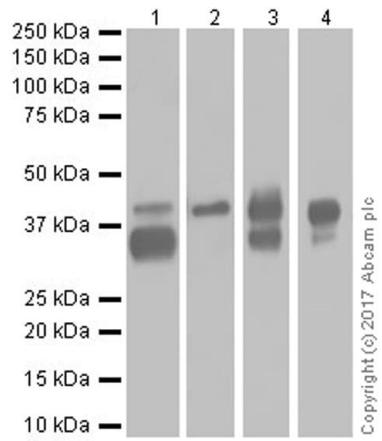
Immunohistochemical analysis of paraffin-embedded human colon tissue labeling EpCAM with ab213500 at 1/16000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

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) (**ab97051**) at 1/500 dilution.

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



Western blot - Anti-EpCAM antibody [EPR20532-222] (ab213500)

All lanes : Anti-EpCAM antibody [EPR20532-222] (ab213500) at 1/2000 dilution

Lane 1 : T-47D (Human ductal breast epithelial tumor cell line) whole cell lysate
Lane 2 : SK-OV-3 (Human ovarian cancer cell line) whole cell lysate
Lane 3 : HT-29 (Human colorectal adenocarcinoma cell line) whole cell lysate
Lane 4 : HCT 116 (Human colorectal carcinoma cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

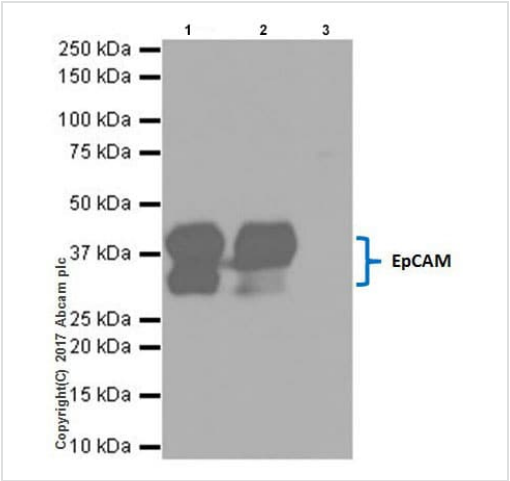
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 35 kDa
Observed band size: 36,40 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1: 15 seconds; Lane 2: 3 minutes; Lane 3: 10 seconds; Lane 4: 15 seconds.

The MW observed is consistent with the literature: PMID19136966; PMID 23618806.



Immunoprecipitation - Anti-EpCAM antibody [EPR20532-222] (ab213500)

EpCAM was immunoprecipitated from 0.35 mg of HCT 116 (Human colorectal carcinoma cell line) whole cell lysate with ab213500 at 1/40 dilution.

Western blot was performed from the immunoprecipitate using ab213500 at 1/1000 dilution.

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

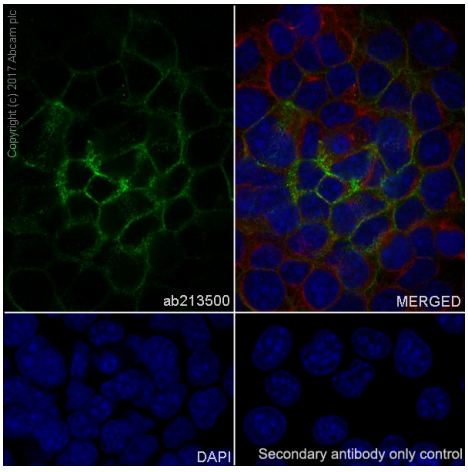
Lane 1: HCT 116 whole cell lysate 10 µg (Input).

Lane 2: ab213500 IP in HCT 116 whole cell lysate.

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab213500 in HCT 116 whole cell lysate.

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

Exposure time: 30 seconds.



Immunocytochemistry/ Immunofluorescence - Anti-EpCAM antibody [EPR20532-222] (ab213500)

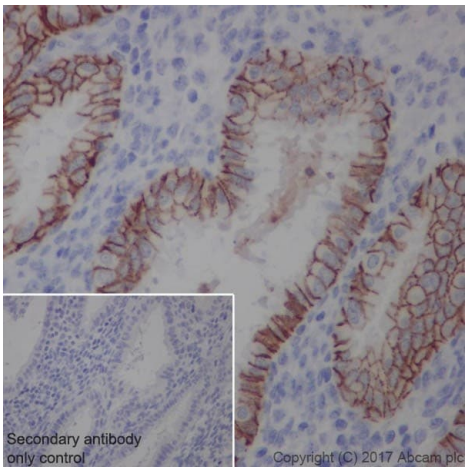
Immunofluorescent analysis of 100% methanol-fixed 4T1 (Mouse mammary gland carcinoma cell line) cells labeling EpCAM with ab213500 at 1/100 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green).

Confocal image showing membranous staining on 4T1 cells.

The nuclear counterstain is DAPI (blue).

Tubulin is detected with **ab195889** (Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594)) at 1/200 dilution (red).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) at 1/1000 dilution.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-EpCAM antibody [EPR20532-222] (ab213500)

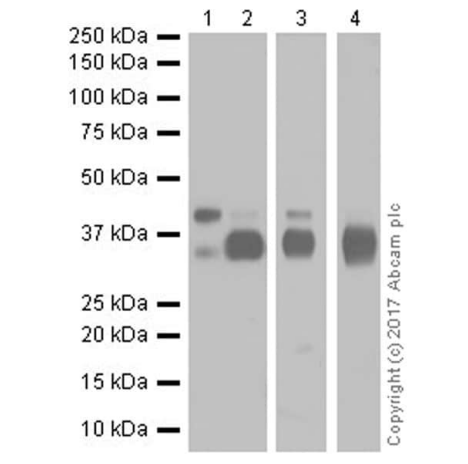
Immunohistochemical analysis of paraffin-embedded human endometrium cancer tissue labeling EpCAM with ab213500 at 1/16000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

Membranous staining on tumor cells of human endometrium 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) (**ab97051**) at 1/500 dilution.

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



Western blot - Anti-EpCAM antibody [EPR20532-222] (ab213500)

All lanes : Anti-EpCAM antibody [EPR20532-222] (ab213500) at 1/2000 dilution

Lane 1 : Human breast cancer lysate
Lane 2 : 4T1 (Mouse mammary gland carcinoma cell line) whole cell lysate
Lane 3 : Human colon lysate
Lane 4 : Mouse small intestine lysate

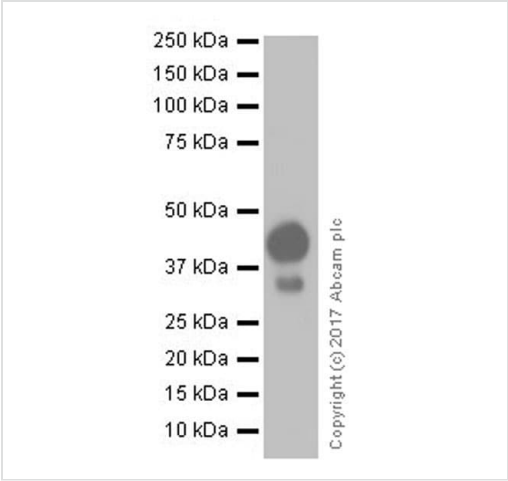
Lysates/proteins at 20 µg per lane.

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

Predicted band size: 35 kDa
Observed band size: 36,40 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1/2: 3 minutes; Lane 3: 10 seconds; Lane 4: 15 seconds.



Western blot - Anti-EpCAM antibody [EPR20532-222] (ab213500)

Anti-EpCAM antibody [EPR20532-222] (ab213500) at 1/1000 dilution + Human fetal kidney lysate at 10 µg

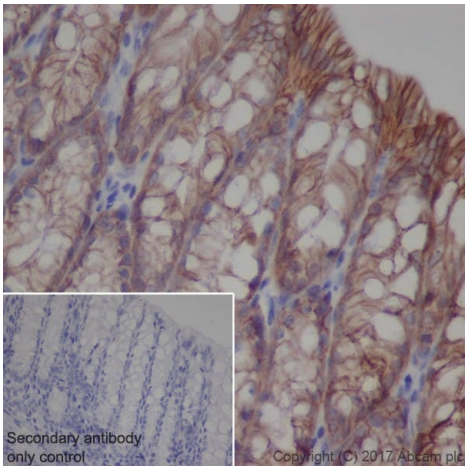
Secondary

VeriBlot for IP Detection Reagent (HRP) (**ab131366**) at 1/4000 dilution

Predicted band size: 35 kDa
Observed band size: 36,40 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-EpCAM antibody [EPR20532-222] (ab213500)

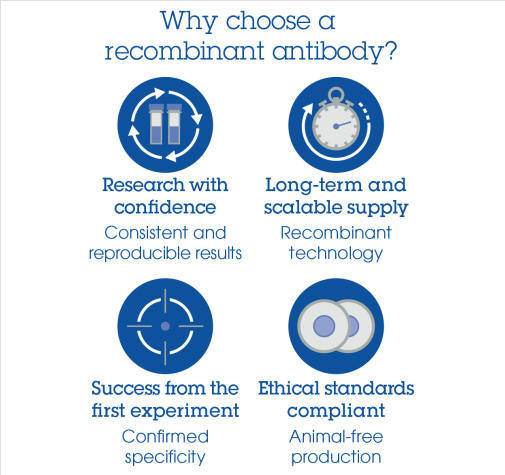
Immunohistochemical analysis of paraffin-embedded mouse colon tissue labeling EpCAM with ab213500 at 1/16000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

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 IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

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



Anti-EpCAM antibody [EPR20532-222] (ab213500)

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