abcam

Product datasheet

Anti-EpCAM antibody [EPR20533-63] ab221552

Recombinant RabMAb

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Overview

Product name	Anti-EpCAM antibody [EPR20533-63]
Description	Rabbit monoclonal [EPR20533-63] to EpCAM
Host species	Rabbit
Tested applications	**Suitable for:** IP, Flow Cyt, ICC/IF, WB, IHC-P
Species reactivity	**Reacts with:** Mouse
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: 4T1 whole cell lysate; Mouse colon, kidney and small intestine lysates. IHC-P: Mouse colon and lung tissues. ICC/IF: 4T1 cells. Flow Cyt: 4T1 cells. IP: Mouse kidney lysate; 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: 40% Glycerol (glycerin, glycerine), 0.05% BSA, PBS
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR20533-63

Isotype IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab221552 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
IP		1/30.
Flow Cyt		1/500. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ICC/IF		1/100.
WB		1/5000. Detects a band of approximately 39, 12 kDa (predicted molecular weight: 35 kDa).
IHC-P	★★★★★ (1)	1/2000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Target

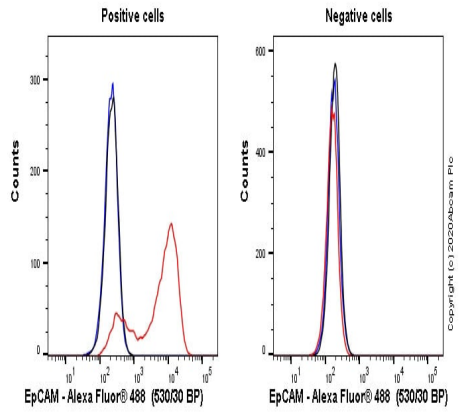
Function May act as a physical homophilic interaction molecule between intestinal epithelial cells (IECs) and intraepithelial lymphocytes (IELs) at the mucosal epithelium for providing immunological barrier as a first line of defense against mucosal infection. Plays a role in embryonic stem cells proliferation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

Tissue specificity 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.

Involvement in disease 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.
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



Flow Cytometry - Anti-EpCAM antibody [EPR20533-63] (ab221552)

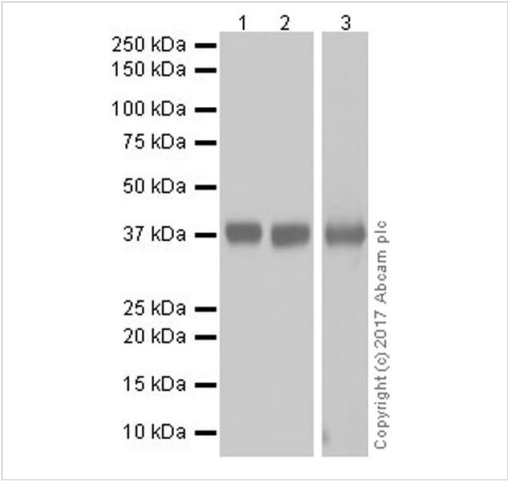
Flow cytometry overlay histogram showing left M158 positive cells and right negative NIH3T3 cells stained with ab221552 (red line). The cells were incubated in 1x PBS containing 10 % normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab221552) ($1x10^6$ in 100 µL at 0.1 µg/ml) for 30 min on ice.

/p>

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

Isotype control antibody (black line) was Rabbit IgG (monoclonal) (**ab172730**) used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control.

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 530/30 bandpass filter.



Western blot - Anti-EpCAM antibody [EPR20533-63] (ab221552)

All lanes : Anti-EpCAM antibody [EPR20533-63] (ab221552) at 1/5000 dilution

Lane 1 : Mouse colon lysate
Lane 2 : Mouse kidney lysate
Lane 3 : 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

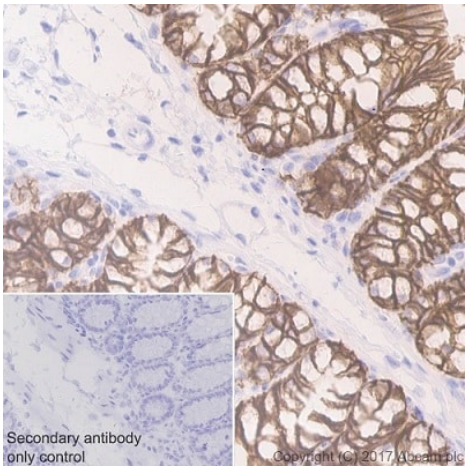
Developed using the ECL technique.

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

Blocking/Dilution buffer: 5% NFDM/TBST.

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

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

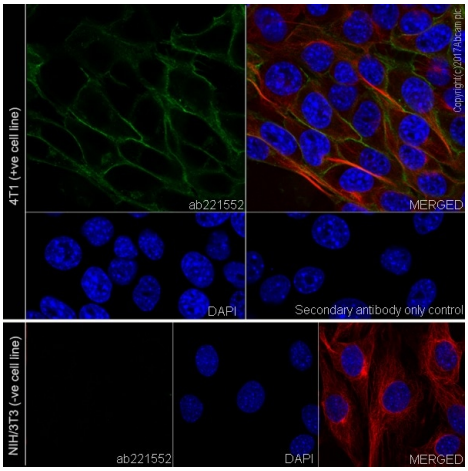


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-EpCAM antibody [EPR20533-63] (ab221552)

Immunohistochemical analysis of paraffin-embedded mouse colon tissue labeling EpCAM with ab221552 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG 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 IgG H&L (HRP) Ready to use.

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



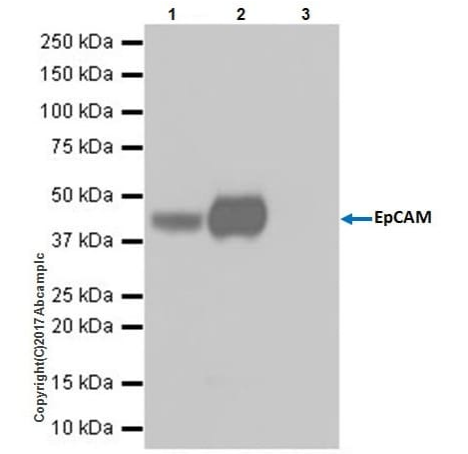
Immunocytochemistry/ Immunofluorescence - Anti-EpCAM antibody [EPR20533-63] (ab221552)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized 4T1 (mouse mammary gland carcinoma cell line) and NIH/3T3 (mouse embryo fibroblast cell line) cells labeling EpCAM with ab221552 at 1/100 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 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 IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution.



Immunoprecipitation - Anti-EpCAM antibody [EPR20533-63] (ab221552)

EpCAM was immunoprecipitated from 0.35 mg of mouse kidney lysate with ab221552 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab221552 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/10000 dilution.

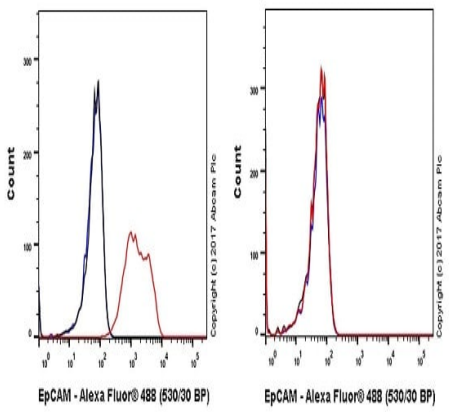
Lane 1: Mouse kidney lysate 10 µg (Input).

Lane 2: ab221552 IP in mouse kidney lysate.

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab221552 in mouse kidney lysate.

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

Exposure time: 3 seconds.

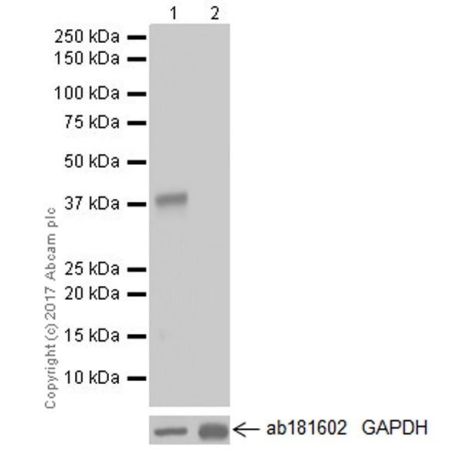


Flow Cytometry - Anti-EpCAM antibody [EPR20533-63] (ab221552)

Flow cytometric analysis of 4% paraformaldehyde-fixed 4T1 (mouse mammary gland carcinoma cell line) and NIH/3T3 (mouse embyro fibroblast cell line) cells labeling EpCAM with ab221552 at 1/500 dilution (red) compared with a Rabbit IgG, monoclonal [EPR25A] - Isotype Control (**ab172730**) (black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1/2000 dilution was used as the secondary antibody.

Flow cytometry was performed with fresh cells without fixation and permeabilization.

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



Western blot - Anti-EpCAM antibody [EPR20533-63] (ab221552)

All lanes : Anti-EpCAM antibody [EPR20533-63] (ab221552) 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 IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Developed using the ECL technique.

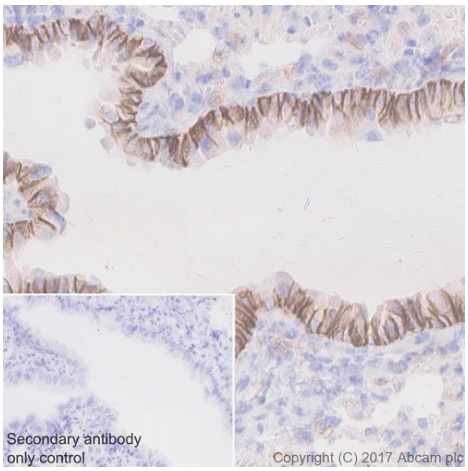
Predicted band size: 35 kDa
Observed band size: 39 kDa

Exposure time: 1 second

Blocking/Dilution buffer: 5% NFDM/TBST.

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

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

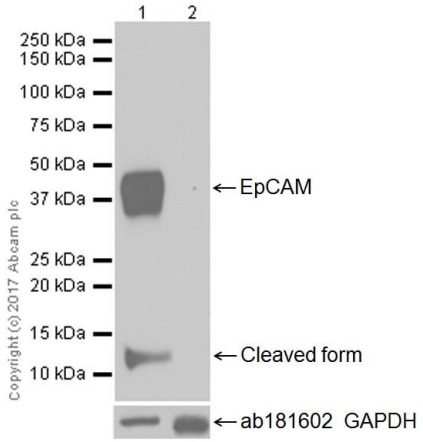


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-EpCAM antibody [EPR20533-63] (ab221552)

Immunohistochemical analysis of paraffin-embedded mouse lung tissue labeling EpCAM with ab221552 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG 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 IgG 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 [EPR20533-63] (ab221552)

All lanes : Anti-EpCAM antibody [EPR20533-63] (ab221552) 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 IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Developed using the ECL technique.

Predicted band size: 35 kDa
Observed band size: 12,39 kDa

Exposure time: 10 seconds

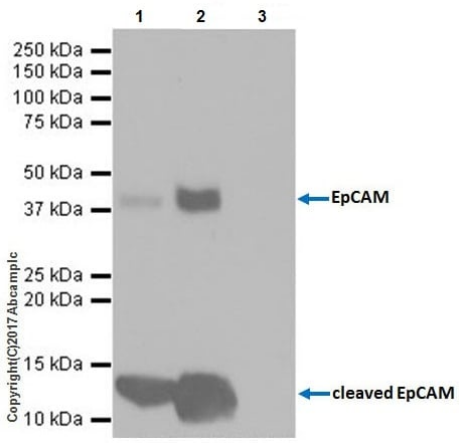
Blocking/Dilution buffer: 5% NFDM/TBST.

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

The band between 12-15kDa has been documented in literature as

the cleaved form of EpCAM (PMID: 23409978).

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



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Lane 1: 4T1 (Mouse mammary gland carcinoma) whole cell lysate 10 µg (Input).

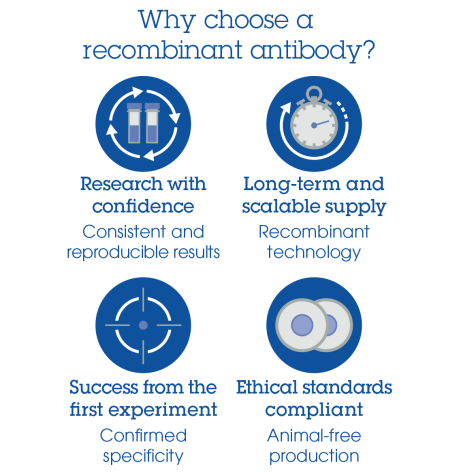
Lane 2: ab221552 IP in 4T1 whole cell lysate .

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab221552 in 4T1 whole cell lysate .

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

Exposure time: 30 seconds.

The band between 12-15kDa has been documented in literature as the cleaved form of EpCAM (PMID: 23409978).



Anti-EpCAM antibody [EPR20533-63] (ab221552)

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