**Product datasheet**

**Anti-EpCAM antibody [EPR20533-63] ab221552**

**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 within Mouse EpCAM aa 1-300. The exact sequence is proprietary. Database link: Q99JW5

**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**

**Storage buffer**
Preservative: 0.01% Sodium azide
Constituents: 40% Glycerol, 0.05% BSA, PBS

**Purity**
Protein A purified

**Clonality**
Monoclonal

**Clone number**
EPR20533-63
Isotype  
IgG

Applications

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.

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<td>1/5000.</td>
<td>Detects a band of approximately 39, 12 kDa (predicted molecular weight: 35 kDa).</td>
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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 applications

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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

Images

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.

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.
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.

**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 embryo 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

why is the actual band size different from the predicted?

**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).

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 why is the actual band size different from the predicted?

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).

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 embryo 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

why is the actual band size different from the predicted?

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).

Flow cytometric analysis of 4% paraformaldehyde-fixed 4T1 (mouse mammary gland carcinoma cell line) and NIH/3T3 (mouse embryo 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).

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.
EpCAM was immunoprecipitated from 0.35 mg of 4T1 (mouse mammary gland carcinoma cell line) whole cell 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: 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).

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