### Overview

**Product name**  
Anti-EpCAM antibody

**Description**  
Rabbit polyclonal to EpCAM

**Host species**  
Rabbit

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

**Species reactivity**  
**Reacts with:** Mouse, Rat, Dog, Human  
**Predicted to work with:** Cow, Pig, Rhesus monkey

**Immunogen**  
Synthetic peptide within Human EpCAM aa 250 to the C-terminus (internal sequence) conjugated to keyhole limpet haemocyanin. The exact sequence is proprietary.  
(Peptide available as ab71915)

**Positive control**  
This antibody gave a positive signal in the following human lysates: HCT 116 Whole Cell, HepG2 Whole Cell, SW480 Whole Cell and Human Ovary Tissue. IHC-P: FFPE human breast adenocarcinoma tissue sections.

### 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 or -80°C. Avoid freeze / thaw cycle.

**Storage buffer**  
- pH: 7.40  
- Preservative: 0.02% Sodium azide  
- Constituent: PBS

Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising agent. If you would like information about the formulation of a specific lot, please contact our scientific support team who will be happy to help.

**Purity**  
Immunogen affinity purified

**Clonality**  
Polyclonal

**Isotype**  
IgG

### Applications
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

Our Abpromise guarantee covers the use of ab71916 in the following tested applications.
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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<tbody>
<tr>
<td>ICC/IF</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use a concentration of 1 - 5 µg/ml.</td>
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<tr>
<td>IHC-P</td>
<td>1/40 - 1/160.</td>
<td></td>
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<tr>
<td>WB</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use a concentration of 1 µg/ml. Detects a band of approximately 35 kDa (predicted molecular weight: 35 kDa).</td>
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Immunocytochemistry / Immunofluorescence - Anti-EpCAM antibody (ab71916)

ab71916 staining EpCam in HT29 cells. The cells were fixed with 100% methanol (5min), 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 ab71916 at 1μg/ml and ab7291, Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control, at 1/1000 dilution. Cells were then incubated with ab150120, Goat polyclonal Secondary Antibody to Mouse at 1/1000 dilution (shown in pseudocolour red) and ab150081, Goat polyclonal Secondary Antibody to Rabbit IgG at 1/1000 dilution (shown in green). Nuclear DNA was labelled with DAPI (shown in blue).

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-EpCAM antibody (ab71916)

IHC image of EpCAM staining in human breast adenocarcinoma formalin fixed paraffin embedded tissue section*, performed on a Leica Bond™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab75962, 1µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

* Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre
Western blot - Anti-EpCAM antibody (ab71916)

All lanes: Anti-EpCAM antibody (ab71916) at 1 µg/ml

Lane 1: Colon (Mouse) Tissue Lysate
Lane 2: Colon (Rat) Tissue Lysate
Lane 3: HCT 116 (Human Colorectal Carcinoma) Whole Cell Lysate
Lane 4: SW480 (Human colon adenocarcinoma cell line) Whole Cell Lysate
Lane 5: Caco 2 (Human colonic carcinoma cell line) Whole Cell Lysate
Lane 6: HuES7 (Human embryonic stem cell line) Whole Cell Lysate
Lane 7: HepG2 (Human hepatocellular liver carcinoma cell line) Whole Cell Lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes: Goat Anti-Rabbit IgG H&L (HRP) preadsorbed (ab97080) at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 35 kDa
Observed band size: 35 kDa
Additional bands at: 40 kDa. We are unsure as to the identity of these extra bands.

Secondary antibody - goat anti-rabbit HRP (H&L preadsorbed; ab97080)
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-EpCAM antibody

ab71916 (1:160) staining EpCAM in paraffin-embedded human breast tissue, using an automated system (Ventana Discovery). Using this protocol there is strong membrane staining of the basolateral membranes of normal breast epithelial cells of breast ducts and lobular acini. There is associated weak to moderate staining of the cytoplasm of these cells.

Sections were rehydrated and antigen retrieved in CC1 Cell Conditioning Buffer using Ventana Standard Retrieval programme. Slides were blocked in 3% H$_2$O$_2$ / 4 min / 37°C and incubated with ab71916 (1:160 dilution / 2 hours / 37°C). Sections then blocked (4mins / 37°C) and incubated with Dako swine anti-rabbit antibody (1:50, 28 min / 37°C). Staining was amplified and detected by incubation with Ventana Streptavidin ABC system (16 min / 37°C) and Ventana DAB map reagent (8 min / 37°C). Slides were counterstained with Haematoxylin and coverslipped in DPX.

For manual

Immunocytochemical immunofluorescence analysis of methanol-fixed rat primary alveolar type II cells, labelling EpCAM with ab71916 at a dilution of 1/100 incubated for 1 hour at 25°C. Permeabilization was with Triton X. Blocking was with 5% BSA incubated for 30 minutes at 25°C. Secondary was a donkey anti-rabbit Alexa Fluor® 568 conjugate at 1/400.
ab71916 (1:40) staining EpCAM in paraffin-embedded human breast carcinoma (Grade 2 Invasive Ductal Carcinoma) using an automated system (Ventana Discovery).

Using this protocol there is moderate to strong membrane staining in carcinoma cells which may be apical or complete instead of basolateral. There is associated moderate to strong staining of the cytoplasm of these cells.

Sections were rehydrated and antigen retrieved in CC1 Cell Conditioning Buffer using Ventana Standard Retrieval programme. Slides were blocked in 3% H₂O₂/4 min / 37°C and incubated with ab71916 (1:40 dilution / 1 hour / 37°C). Sections then blocked (4mins / 37°C) and incubated with Dako swine anti-rabbit antibody (1:50, 28 min / 37°C). Staining was amplified and detected by incubation with Ventana Streptavidin ABC system (16 min / 37°C) and Ventana DAB map reagent (8 min / 37°C). Slides were counterstained with Haematoxylin and coverslipped in DAB.