abcam

Product datasheet

Anti-EpCAM antibody [323/A3] ab85987

KO VALIDATED

10 References 5 Images

Overview

Product name	Anti-EpCAM antibody [323/A3]
Description	Mouse monoclonal [323/A3] to EpCAM
Host species	Mouse
Specificity	We have data to indicate that this antibody may not cross react with Rat. However, this has not been conclusively tested and expression levels may vary in certain cell lines/tissues.
Tested applications	**Suitable for:** ICC/IF, Flow Cyt, WB
Species reactivity	**Reacts with:** Human
Immunogen	Tissue, cells or virus. MCF7 (human breast adenocarcinoma cell line) cells.
Positive control	Flow Cyt: A431 cells. ICC/IF: A431 and T47D cells. WB: MCF-7 cell lysate
General notes	The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid repeated freeze / thaw cycles.
Storage buffer	pH: 7.40 Preservative: 0.097% Sodium azide Constituent: PBS
Purity	Protein A purified
Purification notes	Purified from cell culture supernatant by protein A affinity chromatography, purity > 95% (by SDS-PAGE).
Clonality	Monoclonal
Clone number	323/A3

Isotype IgG1

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab85987 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
ICC/IF		Use a concentration of 1 - 10 µg/ml.
Flow Cyt		Use a concentration of 1 - 4 µg/ml.
WB		Use a concentration of 1 - 2 µg/ml. Use under non reducing condition. Detects a band of approximately 40 kDa (predicted molecular weight: 35 kDa).

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.

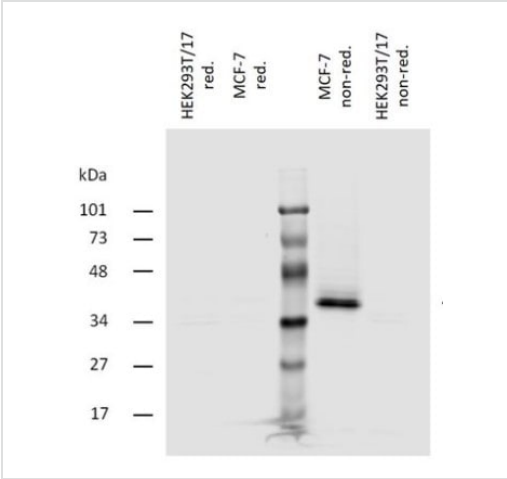
Tissue specificity Highly and selectively expressed by undifferentiated rather than differentiated embryonic stem cells (ESC). Levels rapidly diminish as soon as ESC's differentiate (at protein levels). Expressed in almost all epithelial cell membranes but not on mesodermal or neural cell membranes.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



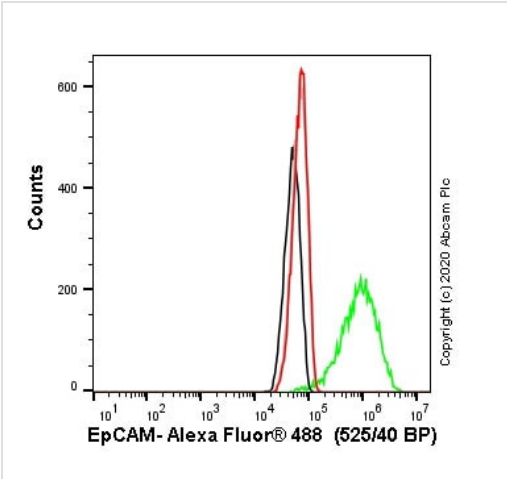
Western blot - Anti-EpCAM antibody [323/A3] (ab85987)

All lanes : Anti-EpCAM antibody [323/A3] (ab85987) at 2 µg/ml

Lane 1 : HEK293T/17 cell lysate (reducing)
Lane 2 : MCF7 cell lysate (reducing)
Lane 3 : HEK293T/17 cell lysate (non-reducing)
Lane 4 : MCF7 cell lysate (non-reducing)

Secondary
All lanes : IRDye800-conjugated anti-mouse IgG1

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



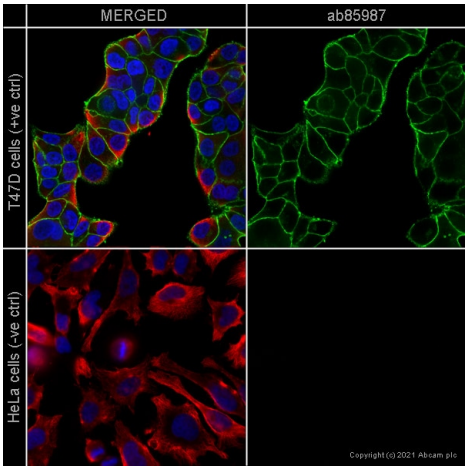
Flow Cytometry - Anti-EpCAM antibody [323/A3] (ab85987)

Flow cytometry overlay histogram showing wild-type A431 (green line) and EPCAM knockout A431 cells stained with ab85987 (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 (ab85987) ($1x10^6$ in 100µl at 0.2 µg/ml) for 30 min at 4°C.

The secondary antibody Goat anti-mouse IgG H&L (Alexa Fluor® 488, pre-adsorbed) (**ab150117**) was used at 1/2000 for 30 min at 4°C.

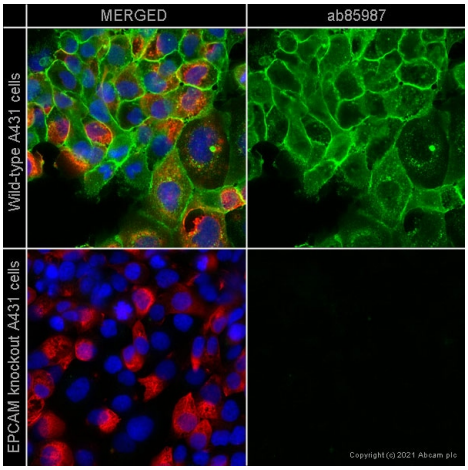
Isotype control antibody was mouse IgG1κ (**ab170190**) used at the same concentration and conditions as the primary antibody (wild-type A431 - black line; EPCAM knockout A431 - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity).

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.



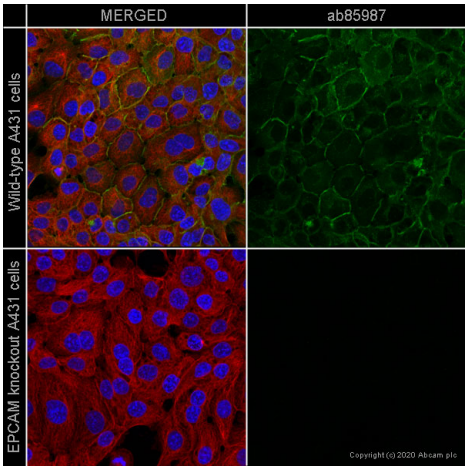
Immunocytochemistry/ Immunofluorescence - Anti-EpCAM antibody [323/A3] (ab85987)

ab85987 staining EpCAM in T47D positive cells (top panel) and HeLa negative cells (bottom panel). The cells were fixed with 4% paraformaldehyde (10 min) then permeabilized with 0.1% PBS-Tween 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 with ab85987 at 0.1µg/ml concentration and **ab6046** (Rabbit polyclonal to beta Tubulin) at 1/1000 dilution overnight at 4°C followed by a further incubation at room temperature for 1h with a goat secondary antibody to mouse IgG (Alexa Fluor® 488) (**ab150117**) at 2 µg/ml (shown in green) and a goat secondary antibody to rabbit IgG (Alexa Fluor® 594) (**ab150080**) at 2 µg/ml (shown in red). Nuclear DNA was labelled in blue with DAPI. This antibody performed similarly using 100% methanol fixation. Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a single confocal section is shown.



Immunocytochemistry/ Immunofluorescence - Anti-EpCAM antibody [323/A3] (ab85987)

ab85987 staining EpCAM in wild-type A431 cells (top panel) and EpCAM knockout A431 cells (bottom panel). The cells were fixed with 4% paraformaldehyde (10 min) then permeabilized with 0.1% PBS-Tween 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 with ab85987 at 0.1µg/ml concentration and **ab6046** (Rabbit polyclonal to beta Tubulin) at 1/1000 dilution overnight at 4°C followed by a further incubation at room temperature for 1h with a goat secondary antibody to mouse IgG (Alexa Fluor® 488) (**ab150117**) at 2 µg/ml (shown in green) and a goat secondary antibody to rabbit IgG (Alexa Fluor® 594) (**ab150080**) at 2 µg/ml (shown in red). Nuclear DNA was labelled in blue with DAPI. This antibody performed similarly using 100% methanol fixation. Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a single confocal section is shown.



Immunocytochemistry/ Immunofluorescence - Anti-EpCAM antibody [323/A3] (ab85987)

ab85987 staining EpCAM in wild-type A431 cells (top panel) and EpCAM knockout A431 cells (bottom panel). The cells were fixed with 100% methanol (5 min) then permeabilized with 0.1% PBS-Tween 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 with ab85987 at 0.5µg/ml concentration and **ab6046** (Rabbit polyclonal to beta Tubulin) at 1/1000 dilution overnight at 4°C followed by a further incubation at room temperature for 1h with a goat secondary antibody to mouse IgG (Alexa Fluor® 488) (**ab150117**) at 2 µg/ml (shown in green) and a goat secondary antibody to rabbit IgG (Alexa Fluor® 594) (**ab150080**) at 2 µg/ml (shown in red). Nuclear DNA was labelled in blue with DAPI. Image was taken with a confocal microscope (Leica-Microsystems TCS SP8).

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