# abcam

# Product datasheet

# Anti-EpCAM antibody [E144] - BSA and Azide free ab183178



Recombinant

RabMAb

# 5 Images

#### Overview

Product name Anti-EpCAM antibody [E144] - BSA and Azide free

**Description** Rabbit monoclonal [E144] to EpCAM - BSA and Azide free

Host species Rabbit

Tested applications Suitable for: WB

Unsuitable for: ICC/IF,IHC-Fr or IP

**Species reactivity** Reacts with: Mouse, Rat, Human

**Immunogen** Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

**Positive control** WB: HCT 116, A431, MCF7, Mouse colon tissue, and Rat colon tissue.

**General notes** ab183178 is the carrier-free version of <u>ab32392</u>.

Our <u>carrier-free</u> antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.

This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our <u>conjugation kits</u> for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

This product is compatible with the Maxpar<sup>®</sup> Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar<sup>®</sup> is a trademark of Fluidigm Canada Inc.

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<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**<sup>®</sup> **patents**.

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# **Properties**

Form Liquid

**Storage instructions** Shipped at 4°C. Store at +4°C. Do Not Freeze.

Storage buffer pH: 7.2

Constituent: PBS

Carrier free Yes

Purity Protein A purified

**Clonality** Monoclonal

Clone number E144
Isotype IgG

#### **Applications**

#### The Abpromise guarantee

Our Abpromise quarantee covers the use of ab183178 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
WB		Use at an assay dependent concentration. Predicted molecular weight: 39 kDa.

# Application notes

Is unsuitable for ICC/IF,IHC-Fr or IP.

#### **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 extracolonic 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

 $\label{thm:linear} \mbox{Hyperglycosylated in carcinoma tissue as compared with autologous normal epithelia.}$ 

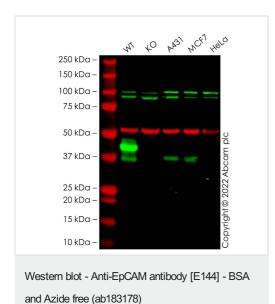
Glycosylation at Asn-198 is crucial for protein stability.

**Cellular localization** 

 $\label{lem:lembrane} Lateral\ cell\ membrane.\ Cell\ junction > tight\ junction.\ Co-localizes\ with\ CLDN7\ at\ the\ lateral\ cell$ 

membrane and tight junction.

#### **Images**



**All lanes :** Anti-EpCAM antibody [E144] (<u>ab32392</u>) 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:** 39 kDa **Observed band size:** 37-45 kDa

False colour image of Western blot: Anti-EpCAM antibody [E144] 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, ab32392 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<sup>®</sup> 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<sup>®</sup> 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye<sup>®</sup> 680RD) preabsorbed (ab216776) at 1/20000 dilution.

1 2 3

250 kDa = 150 kDa = 100 kDa = 75 kDa = 25 kDa = 20 kDa = 15 kDa = 10 kDa = 10

Western blot - Anti-EpCAM antibody [E144] - BSA and Azide free (ab183178)

**All lanes :** Anti-EpCAM antibody [E144] (<u>ab32392</u>) at 1/1000 dilution

Lane 1: Mouse colon tissue lysate

Lane 2: Rat small intestine tissue lysate

Lane 3: Rat colon tissue lysate

Lysates/proteins at 20 µg per lane.

# Secondary

**All lanes :** Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution (Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated)

Predicted band size: 39 kDa

250 kDa - 150 kD

Western blot - Anti-EpCAM antibody [E144] - BSA and Azide free (ab183178)

**All lanes :** Anti-EpCAM antibody [E144] (<u>ab32392</u>) at 1/2000 dilution

Lane 1: Wild-type A431 cell lysate

Lane 2: EPCAM knockout A431 cell lysate

Lane 3: MCF7 (Human breast adenocarcinoma cell line) whole

cell lysate

Lane 4: HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

**Predicted band size:** 39 kDa **Observed band size:** 40 kDa

This data was developed using the same antibody clone in a different buffer formulation (ab32392).

**Lanes 1 - 4:** Merged signal (red and green). Green - <u>ab32392</u> observed at 40 kDa. Red - loading control <u>ab7291</u> (Mouse anti-Alpha Tubulin [DM1A] observed at 55kDa.

ab32392 was shown to react with EpCAM in wild-type A431 cells in western blot with loss of signal observed in EpCAM knockout sample. Wild-type and EpCAM knockout A431 cell lysates were subjected to SDS-PAGE. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with ab32392 and ab7291 (Mouse anti-Alpha Tubulin [DM1A] overnight at 4°C at a 1 in 2000 dilution and a 1 in 20000 dilution respectively. Blots were incubated 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.

\$0a 250-150-100-75-50-37-25-20-16-

Western blot - Anti-EpCAM antibody [E144] - BSA and Azide free (ab183178)

Anti-EpCAM antibody [E144] (ab32392) at 1/2500 dilution + A431 cell lysate

Predicted band size: 39 kDa

This data was developed using <u>ab32392</u>, the same antibody clone in a different buffer formulation.



(ab183178)

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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