# abcam

### Product datasheet

# Anti-EpCAM antibody [EPR20533-63] - BSA and Azide free ab228876



#### Overview

Product name Anti-EpCAM antibody [EPR20533-63] - BSA and Azide free

**Description** Rabbit monoclonal [EPR20533-63] to EpCAM - BSA and Azide free

Host species Rabbit

Tested applications Suitable for: WB, ICC/IF, Flow Cyt, IP, IHC-P

Species reactivity Reacts with: Mouse

**Immunogen** Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.

Positive control IHC-P: Mouse colon tissue.

**General notes** ab228876 is the carrier-free version of <u>ab221552</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 **conjugation kits** 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**.

1

#### **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 EPR20533-63

**Isotype** IgG

#### **Applications**

The Abpromise guarantee Our Abpromise guarantee covers the use of ab228876 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. Detects a band of approximately 39, 12 kDa (predicted molecular weight: 35 kDa).
ICC/IF		Use at an assay dependent concentration.
Flow Cyt		Use at an assay dependent concentration. <u>ab199376</u> - Rabbit monoclonal lgG (Low endotoxin, Azide free), is suitable for use as an isotype control with this antibody.
IP		Use at an assay dependent concentration.
IHC-P	****(1)	Use at an assay dependent concentration. 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 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

Post-translational modifications

**Cellular localization** 

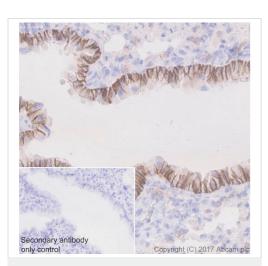
Belongs to the EPCAM family.

Contains 1 thyroglobulin type-1 domain.

Hyperglycosylated in carcinoma tissue as compared with autologous normal epithelia. Glycosylation at Asn-198 is crucial for protein stability.

Lateral cell membrane. Cell junction > tight junction. Co-localizes with CLDN7 at the lateral cell membrane and tight junction.

#### **Images**



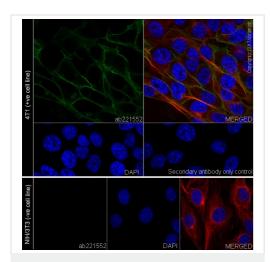
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-EpCAM antibody
[EPR20533-63] - BSA and Azide free (ab228876)

Immunohistochemical analysis of paraffin-embedded mouse lung tissue labeling EpCAM with <u>ab221552</u> 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.

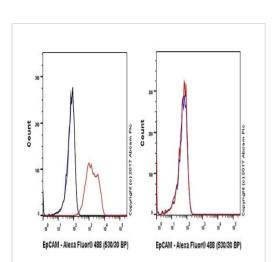
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) Ready to use.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab221552).

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] - BSA and Azide free (ab228876)



Flow Cytometry - Anti-EpCAM antibody [EPR20533-63] - BSA and Azide free (ab228876)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized 4T1 (mouse mammary gland carcinoma cell line) and NIH/3T3 (mouse embyro fibroblast cell line) cells labeling EpCAM with <a href="mailto:ab221552">ab221552</a> at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (<a href="mailto:ab150077">ab150077</a>) 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<sup>®</sup> 594) (**ab195889**) (red) at 1/200 dilution.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (Alexa Fluor<sup>®</sup> 488) (ab150077) secondary antibody at 1/1000 dilution.

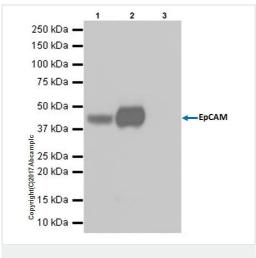
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab221552).

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 <u>ab221552</u> at 1/500 dilution (red) compared with a Rabbit lgG, monoclonal [EPR25A] - Isotype Control (<u>ab172730</u>) (black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat Anti-Rabbit lgG H&L (Alexa Fluor<sup>®</sup> 488) at 1/2000 dilution was used as the secondary antibody.

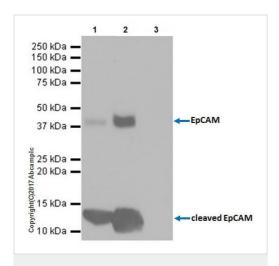
Flow cytometry was performed with fresh cells without fixation and permeabilization.

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab221552).



Immunoprecipitation - Anti-EpCAM antibody
[EPR20533-63] - BSA and Azide free (ab228876)



Immunoprecipitation - Anti-EpCAM antibody
[EPR20533-63] - BSA and Azide free (ab228876)

EpCAM was immunoprecipitated from 0.35 mg of mouse kidney lysate with <u>ab221552</u> at 1/30 dilution. Western blot was performed from the immunoprecipitate using <u>ab221552</u> at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>), 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  $\lg G$  ( $\underline{ab172730}$ ) instead of  $\underline{ab221552}$  in mouse kidney lysate.

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

Exposure time: 3 seconds.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab221552).

EpCAM was immunoprecipitated from 0.35 mg of 4T1 (mouse mammary gland carcinoma cell line) whole cell lysate with <a href="mailto:ab221552">ab221552</a> at 1/30 dilution. Western blot was performed from the immunoprecipitate using <a href="mailto:ab221552">ab221552</a> at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (<a href="mailto:ab131366">ab131366</a>), was used for detection at 1/10000 dilution.

Lane 1: 4T1 (Mouse mammary gland carcinoma) whole cell lysate  $10 \mu g$  (Input).

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

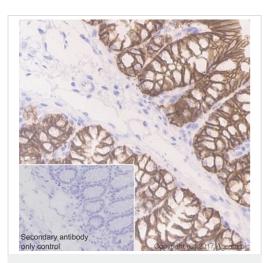
Lane 3: Rabbit monoclonal  $\lg G$  (<u>ab172730</u>) instead of <u>ab221552</u> in 4T1 whole cell lysate .

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).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab221552</u>).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-EpCAM antibody

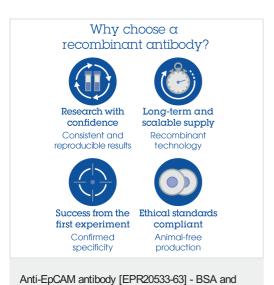
[EPR20533-63] - BSA and Azide free (ab228876)

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab221552).

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



Azide free (ab228876)

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

#### Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit <a href="https://www.abcam.com/abpromise">https://www.abcam.com/abpromise</a> or contact our technical team.

## Terms and conditions

• Guarantee only valid for products bought direct from Abcam or one of our authorized distributors