abcam

Product datasheet

Anti-CEACAM1 antibody [EPR26490-88] ab300061



Recombinant RabMAb

11 Images

Overview

Product name Anti-CEACAM1 antibody [EPR26490-88]

Description Rabbit monoclonal [EPR26490-88] to CEACAM1

Host species Rabbit

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

Unsuitable for: IHC-Fr

Reacts with: Human Species reactivity

Does not react with: Mouse, Rat

Recombinant fragment corresponding to Human CEACAM1 aa 1-450. **Immunogen**

Database link: P13688

Run BLAST with Run BLAST with

Positive control WB: Wild-type A549, HepG2, Human kidney, Human colon, A-204, SW480, His-tagged human

> CEACAM-1 recombinant protein aa35-428 recombinant protein lysates. IHC-P: Human: colon, endometrium, liver and hepatic carcinoma tissues ICC/IF: A549 cells. Flow cyt.: Wild-type A549

cell. IP: A549 cell.

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 Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long

term. Avoid freeze / thaw cycle.

Storage buffer pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

Purity Protein A purified

Clonality Monoclonal
Clone number EPR26490-88

Isotype IgG

Applications

The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab300061 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
Flow Cyt		1/500.
IHC-P		1/2000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		1/1000. Predicted molecular weight: 57 kDa.
IP		1/30.
ICC/IF		1/500.

Application notes

Is unsuitable for IHC-Fr.

Target

Sequence similarities Belongs to the immunoglobulin superfamily. CEA family.

Contains 3 lg-like C2-type (immunoglobulin-like) domains. Contains 1 lg-like V-type (immunoglobulin-like) domain.

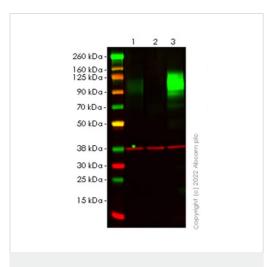
Cellular localization Secreted; Cell membrane and Cell membrane. Localizes to sites of cell-cell contact.

Form There are 11 isoforms produced by alternative splicing. Isoform 1 = BGPa; CEACAM1-4L; TM1-

CEA; Isoform 2 = BGPg; CEACAM1-4C1; Isoform 3 = BGPh; CEACAM1-3; Isoform 4 = BGPi; CEACAM1-3C2; Isoform 5 = BGPy; CEACAM1-3AL; Isoform 6 = BGPb; CEACAM1-3L; TM2-CEA; Isoform 7 = BGPx; CEACAM1-1L; Isoform 8 = BGPc; CEACAM1-4S; TM3-CEA; Isoform 9

= BGPz; CEACAM1-3AS and Isoform 11 = BGPd; CEACAM1-3S.

Images



Western blot - Anti-CEACAM1 antibody [EPR26490-88] (AB300061)

All lanes : Anti-CEACAM1 antibody [EPR26490-88] (ab300061) at 1/1000 dilution

Lane 1: Wild-type A549 (ab255450) whole cell lysate

Lane 2: CEACAM1 knockout A549 (ab267047) whole cell lysate

Lane 3 : HepG2 (human hepatocellular carcinoma epithelial cell)

whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

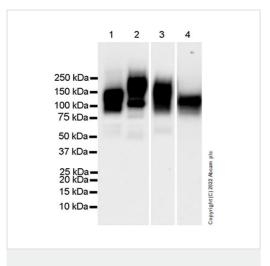
All lanes : Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (<u>ab216776</u>) at 1/10000 dilution

Predicted band size: 57 kDa

Observed band size: 100-180 kDa

Lanes 1-3: Merged signal (red and green). Green - ab300061 observed at 100-180 kDa. Red - loading control ab8245 observed at 36 kDa.

ab300061 Anti-CEACAM1 antibody [EPR26490-88] was shown to specifically react with CEACAM1 in wild-type A549 cells (ab255450). Loss of signal was observed when knockout cell line (ab267047) was used. Wild-type and CEACAM1 knockout samples were subjected to SDS-PAGE. ab300061 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated at 4? overnight at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1 in 10000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-CEACAM1 antibody [EPR26490-88] (AB300061)

All lanes : Anti-CEACAM1 antibody [EPR26490-88] (ab300061) at 1/1000 dilution

Lane 1: Human kidney tissue lysate

Lane 2: Human colon tissue lysate

Lane 3: A-204 (human muscle rhabdomyosarcoma) whole cell

lysate

Lane 4: SW480 (human colorectal adenocarcinoma epithelial cell)

whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

Lanes 1-2: Goat Anti-Rabbit IgG (HRP) with minimal cross-

reactivity with human lgG (Merck DC03L)

 $\textbf{Lanes 3-4:} \ \, \textbf{Goat Anti-Rabbit lgG H\&L (HRP) (} \underline{\textbf{ab97051}} \textbf{)} \ \, \textbf{at}$

1/20000 dilution

Predicted band size: 57 kDa

Observed band size: 100-180 kDa

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

Exposure time: Lanes 1-3:10 seconds; Lane 4: 48 seconds.

All lanes : Anti-CEACAM1 antibody [EPR26490-88] (ab300061) at 1/1000 dilution

Lane 1 : His-tagged human CEACAM-1 recombinant protein aa (aa35-428) 20ng

Lane 2: His-tagged human CEACAM-5/CD66e recombinant protein (aa35-685) 20ng

Lane 3: His-tagged human CEACAM-6/CD66c recombinant protein (aa35-320) 20ng

Lane 4: His-tagged human CEACAM-8/CD66b recombinant protein(aa35-320) 20ng

1 2 3 4 250 KDa— 150 KDa— 150 KDa— 75 KDa— 50 KDa— 37 KDa— 25 KDa— 20 KDa— 15 KDa— 150 KDa— 150 KDa— 175 KDa—

Western blot - Anti-CEACAM1 antibody [EPR26490-88] (AB300061)

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/20000 dilution

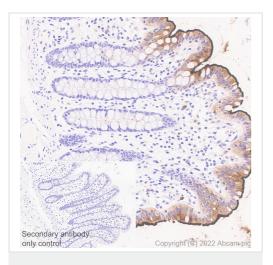
Predicted band size: 57 kDa

Observed band size: 100-180 kDa

Exposure time: 48 seconds

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

Exposure time: Lanes 1-3:10 seconds; Lane 4: 48 seconds.

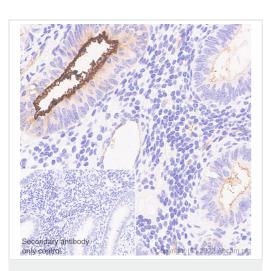


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CEACAM1 antibody
[EPR26490-88] (AB300061)

Immunohistochemical analysis of paraffin-embedded human colon tissue labelling CEACAM1 with ab300061 at 1/2000 followed by a ready to use LeicaDS9800 (BOND™ Polymer Refine Detection). Positive staining on human colon (PMID: 8543780). The section was incubated with ab300061 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: PBS was used instead of primary antibody (ab300061) followed by ready to use secondary antibody LeicaDS9800 (BOND™ Polymer Refine Detection).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

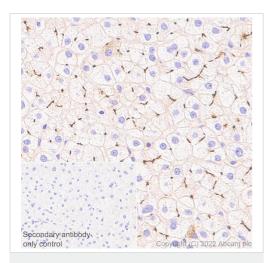


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CEACAM1 antibody
[EPR26490-88] (AB300061)

Immunohistochemical analysis of paraffin-embedded human endometrium tissue labelling CEACAM1 with ab300061 at 1/2000 followed by a ready to use LeicaDS9800 (BOND™ Polymer Refine Detection). Positive staining on human endometrium (PMID: 9626043). The section was incubated with ab300061 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: PBS was used instead of primary antibody (ab300061) followed by ready to use secondary antibody LeicaDS9800 (BOND™ Polymer Refine Detection).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

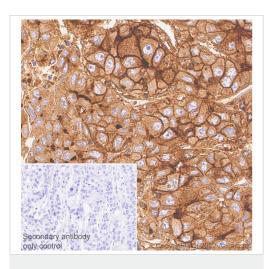


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CEACAM1 antibody
[EPR26490-88] (AB300061)

Immunohistochemical analysis of paraffin-embedded human liver tissue labelling CEACAM1 with ab300061 at 1/2000 followed by a ready to use LeicaDS9800 (BOND™ Polymer Refine Detection). Positive staining on human liver (PMID: 27377843). The section was incubated with ab300061 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: PBS was used instead of primary antibody (ab300061) followed by ready to use secondary antibody LeicaDS9800 (BOND™ Polymer Refine Detection).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

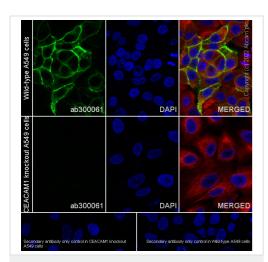


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CEACAM1 antibody
[EPR26490-88] (AB300061)

Immunohistochemical analysis of paraffin-embedded human hepatic carcinoma tissue labelling CEACAM1 with ab300061 at 1/2000 followed by a ready to use LeicaDS9800 (BOND™ Polymer Refine Detection). Positive staining on human hepatic carcinoma (PMID: 27377843). The section was incubated with ab300061 for 30 mins at room temperature The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: PBS was used instead of primary antibody (ab300061) followed by ready to use secondary antibody LeicaDS9800 (BOND™ Polymer Refine Detection).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

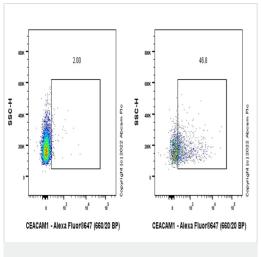


Immunocytochemistry/ Immunofluorescence - Anti-CEACAM1 antibody [EPR26490-88] (ab300061)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeilized CEACAM1 KO A549 (CEACAM1 knockout human lung carcinoma epithelial cell) (ab267047) cells lebelling CEACAM1 with ab300061 at 1/500 dilution, followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 dilution (Green). Confocal image showing membrane and cytoplasmic staining in parental A549 cells, no staining in CEACAM1 KO A549 cells.

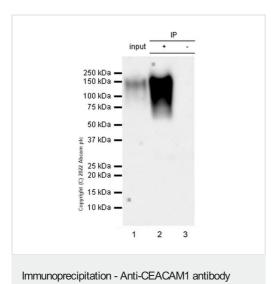
ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor[®] 594) was used to counterstain tubulin at 1/200 dilution (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution.



Flow Cytometry - Anti-CEACAM1 antibody [EPR26490-88] (AB300061)

Flow cytometric analysis of Wild-type A549 (Human lung carcinoma epithelial cell, Right)/ CEACAM1 knockout A549 cells (Left) cells labelling CEACAM1 with ab300061 at 1/500 dilution/ Left and Right compared with a isotype control. Goat anti-Rabbit IgG (Alexa Fluor® 647, <u>ab150083</u>) at 1/2000 dilution was used as the secondary antibody. Gated on viable cells. Positive staining on A549 cells (<u>ab255450</u>), while no staining on CEACAM1 knockout A549 cells (<u>ab267047</u>).



[EPR26490-88] (AB300061)

CEACAM1 was immunoprecipitated from 0.35 mg A549 (human lung carcinoma epithelial cell) whole cell lysate 10 µg with ab300061 at 1/30 dilution. Western blot was performed on the immunoprecipitate using ab300061 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP)(ab131366) was used at 1/5000 dilution.

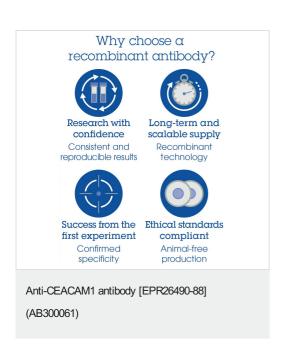
Lane 1: A549 (human lung carcinoma epithelial cell) whole cell lysate 10 μg (lnput)

Lane 2: ab300061 IP in A549 whole cell lysate

Lane 3: Rabbit monoclonal $\lg G \left(\underbrace{ab172730} \right)$ instead of ab300061 in A549 whole cell lysate

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

Exposure time: 32 seconds



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