# abcam

## Product datasheet

# Anti-CEACAM5 + CD66b + CEACAM1 + CEACAM6 antibody [EPR20721] ab207718

Recombinant RabMAb

## 1 References 10 Images

Overview

Product name Anti-CEACAM5 + CD66b + CEACAM1 + CEACAM6 antibody [EPR20721]

**Description** Rabbit monoclonal [EPR20721] to CEACAM5 + CD66b + CEACAM1 + CEACAM6

Host species Rabbit

Specificity ab207718 cross-reacts with CEACAM 1, 6 and 8. There are other CEACAM family members but

we only examined cross-reactivity with those having >55% homology with CEACAM5 following a

BLAST search with the immunogen.

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

Species reactivity Reacts with: Human

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

Positive control WB: His-GST-tagged human CEACAM5 recombinant protein (aa238-686), His-tagged human

CEACAM6 recombinant protein (aa35-326), CEACAM8 recombinant protein (aa35-335) and CEACAM1 recombinant protein (aa35-428). MCF7, LoVo and Caco-2 whole cell lysate. Human colon lysate. IHC-P: Human colon and colon cancer tissue. Human spleen tissue, HEK-293T cells transfected with a CEACAM3, CEACAM5 and CEACAM8 expression vector containing a his

tag. ICC/IF, Flow Cyt: MCF7 cells.

**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<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**.

#### **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.

Avoid freeze / thaw cycle.

Storage buffer pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: PBS, 40% Glycerol, 0.05% BSA

Purity Protein A purified

ClonalityMonoclonalClone numberEPR20721

**Isotype** IgG

#### **Applications**

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab207718 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		1/1000. Predicted molecular weight: 76 kDa.
IHC-P		1/5000 - 1/40000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		1/500.
Flow Cyt		1/500.

# **Target**

CEACAM5: Cell membrane. CD66b: Cell membrane. CEACAM1: Secreted; Cell membrane and

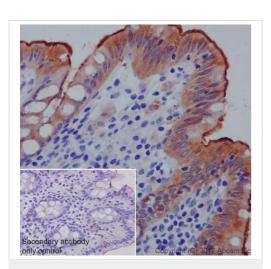
Cell membrane. Localizes to sites of cell-cell contact. CEACAM6: Cell membrane.

**Form** CEACAM1: There are 11 isoforms produced by alternative splicing. Isoform 1 = BGPa;

 $\label{eq:ceacam1-4L} CEACAM1-4L; TM1-CEA; Isoform 2 = BGPg; CEACAM1-4C1; Isoform 3 = BGPh; CEACAM1-3; Isoform 4 = BGPi; CEACAM1-3C2; Isoform 5 = BGPy; CEACAM1-3AL; Isoform 6 = BGPb; CEACAM1-3L; TM2-CEA; Isoform 7 = BGPx; CEACAM1-1L; Isoform 8 = BGPc; CEACAM1-4S; \\$ 

TM3-CEA; Isoform 9 = BGPz; CEACAM1-3AS and Isoform 11 = BGPd; CEACAM1-3S.

#### **Images**

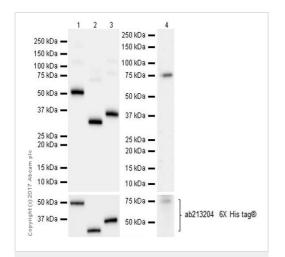


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Carcino Embryonic Antigen CEA antibody [EPR20721] (ab207718)

Immunohistochemical analysis of paraffin-embedded human colon tissue labeling Carcino Embryonic Antigen CEA with ab207718 at 1/40,000 dilution, followed by a ready to use Goat Anti-Rabbit lgG H&L (HRP). Positive staining in human colon (PMID: 25042385) is observed. Counter stained with Hematoxylin.

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

Perform heat mediated antigen retrieval using <u>ab93684</u> (Tris/EDTA buffer, pH 9.0).



Western blot - Anti-Carcino Embryonic Antigen CEA antibody [EPR20721] (ab207718)

**All lanes**: Anti-CEACAM5 + CD66b + CEACAM1 + CEACAM6 antibody [EPR20721] (ab207718) at 1/1000 dilution

**Lane 1 :** His-GST-tagged human CEACAM5 recombinant protein (aa238-686) 10 ng

**Lane 2 :** His-tagged human CEACAM6 recombinant protein (aa35-326) 10 ng

**Lane 3**: His-tagged human CEACAM8 recombinant protein (aa35-335) 10 ng

Lane 4: His-tagged human CEACAM1 recombinant protein (aa35-428) 10 ng

#### Secondary

**All lanes :** Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/100000 dilution

Developed using the ECL technique.

Predicted band size: 76 kDa

Observed band size: 30,50,75 kDa

Additional bands at: 32 kDa (possible post-translational

modification)

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

Exposure times:

Lanes 1-3: 10 seconds

Lane 4: 4 minutes

Observed MW: 50, 30, 32, 75 kDa.

The blot was developed on a BIO-RAD<sup>®</sup> ChemiDoc™ MP

instrument.

All lanes: Anti-CEACAM5 + CD66b + CEACAM1 + CEACAM6 antibody [EPR20721] (ab207718) at 1/1000 dilution

**Lane 1 :** MCF7 (human breast adenocarcinoma epithelial cell), whole cell lysate

Lane 2: PANC-1 (human pancreatic epithelioid carcinoma epithelial cell), whole cell lysate

Lysates/proteins at 20 µg per lane.

ze 25 kDa — 4 20 kDa — 20 kDa — 20 kDa — 20 kDa — ← ab181602 GAPDH

250 kDa — 150 kDa — 100 kDa —

75 kDa -

50 kDa —

Western blot - Anti-Carcino Embryonic Antigen CEA antibody [EPR20721] (ab207718)

#### **Secondary**

**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

Developed using the ECL technique.

Predicted band size: 76 kDa

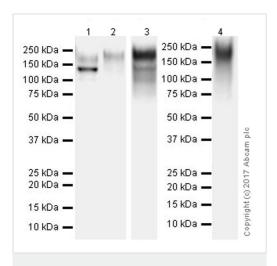
Observed band size: 110-150,75-90 kDa

Exposure time: 58 seconds

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

Observed MW: 110-150, 75-90 kDa.

The blot was developed on a BIO-RAD<sup>®</sup> ChemiDoc™ MP instrument.



Western blot - Anti-Carcino Embryonic Antigen CEA antibody [EPR20721] (ab207718)

**All lanes**: Anti-CEACAM5 + CD66b + CEACAM1 + CEACAM6 antibody [EPR20721] (ab207718) at 1/1000 dilution

**Lane 1 :** Caco-2 (human colorectal adenocarcinoma epithelial cell), whole cell lysate at 10 µg

Lane 2 : SW480 (human colorectal adenocarcinoma epithelial cell), whole cell lysate at 10 µg

Lane 3: Human colon lysate at 20 µg

**Lane 4 :** LoVo (human colorectal adenocarcinoma epithelial cell), whole cell lysate at 10  $\mu g$ 

#### **Secondary**

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

Developed using the ECL technique.

Predicted band size: 76 kDa

Observed band size: 110-150,75-90 kDa

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

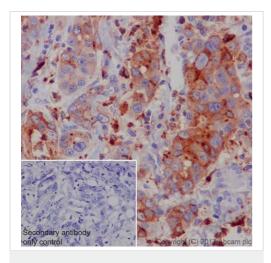
#### **Exposure times:**

Lanes 1-2: 3 minutes

Lanes 3-4: 10 seconds

Observed MW 110-150, 75-90 kDa.

The blot was developed on a BIO-RAD<sup>®</sup> ChemiDoc™ MP instrument. The expression profile observed is consistent with differential glycosylation of this protein (PMID: 1717152).

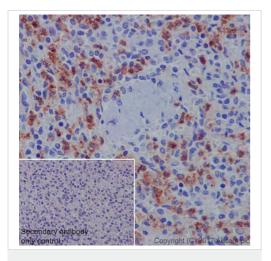


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Carcino Embryonic
Antigen CEA antibody [EPR20721] (ab207718)

Immunohistochemical analysis of paraffin-embedded human colon cancer tissue labeling Carcino Embryonic Antigen CEA with ab207718 at 1/40,000 dilution, followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Positive staining in human colon cancer (PMID: 25042385) is observed. Counter stained with Hematoxylin.

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

Perform heat mediated antigen retrieval using <u>ab93684</u> (Tris/EDTA buffer, pH 9.0).

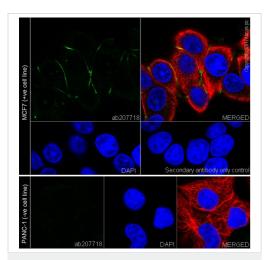


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Carcino Embryonic
Antigen CEA antibody [EPR20721] (ab207718)

Immunohistochemical analysis of paraffin-embedded human spleen tissue labeling Carcino Embryonic Antigen CEA with ab207718 at 1/40,000 dilution, followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Positive staining of spleen-infiltrating neutrophils (PMID: 22064717 and 19077207) is observed. Counter stained with Hematoxylin.

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

Perform heat mediated antigen retrieval using <u>ab93684</u> (Tris/EDTA buffer, pH 9.0).



Immunocytochemistry/ Immunofluorescence - Anti-Carcino Embryonic Antigen CEA antibody [EPR20721] (ab207718)

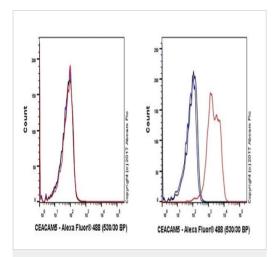
Immunofluorescent analysis of 100% methanol fixed MCF7 (human breast adenocarcinoma epithelial cell) cells labeling Carcino Embryonic Antigen CEA with ab207718 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup> 488) (ab150077) secondary antibody at 1/1000 dilution (green).

Confocal image showing membranous staining on MCF7 cell line.

Negative control for cells: PANC-1.

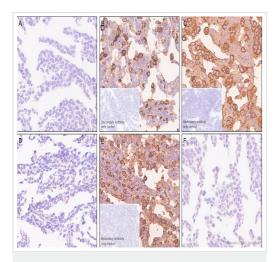
Counterstained with <u>ab195889</u> anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor<sup>®</sup> 594) at a 1/200 dilution (red) and DAPI (blue).

The negative control is the secondary antibody only.



Flow Cytometry - Anti-Carcino Embryonic Antigen CEA antibody [EPR20721] (ab207718)

Flow cytometric analysis of PANC-1 (human pancreatic epithelioid carcinoma epithelial cell) (Left) / MCF7 (human breast adenocarcinoma epithelial cell) (Right) labeling Carcino Embryonic Antigen CEA with ab207718 at 1/500 (red) compared with a Rabbit monoclonal IgG (ab172730) (black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat anti rabbit IgG (Alexa Fluor<sup>®</sup> 488, ab150077), at 1/2000 dilution was used as the secondary antibody. Cells were not fixed or permeabilized. Gated on total viable cells.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CEACAM5 + CD66b + CEACAM1 + CEACAM6 antibody [EPR20721] (ab207718)

Immunohistochemistry analysis of paraffin-embedded (A) HEK-293T cells transfected with a CEACAM1 expression vector containing a his tag, (B) HEK-293T cells transfected with a CEACAM3 expression vector containing a his tag, (C) HEK-293T cells transfected with a CEACAM5 expression vector containing a his tag, (D) HEK-293T cells transfected with a CEACAM6 expression vector containing a his tag, (E) HEK-293T cells transfected with a CEACAM8 expression vector containing a his tag, (F) HEK-293T cells transfected with empty vector containing a his tag, sections labelling CEACAM5 + CD66b + CEACAM1 + CEACAM6 with ab207718 at 1/5000 dilution. The section was incubated with ab207718 for 30 mins at room temperature. LeicaDS9800 (Bond™ Polymer Refine Detection) was used as the secondary antibody. Sections were counterstained with Hematoxylin. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins. Positive staining on (B) HEK-293T cells transfected with a CEACAM3 expression vector containing a his tag, (C) HEK-293T cells transfected with a CEACAM5 expression vector containing a his tag, (E) HEK-293T cells transfected with a CEACAM8 expression vector containing a his tag. Nearly no staining on (A) HEK-293T cells transfected with a CEACAM1 expression vector containing a his tag, (D) HEK-293T cells transfected with a CEACAM6 expression vector containing a his tag and (F) HEK-293T cells transfected with empty vector containing a his tag. The immunostaining was performed on a Leica Biosystems BOND® RX



[EPR20721] (ab207718)

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

instrument.

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