abcam

Product datasheet

Anti-Claudin 4 antibody [EPRR17575] ab210796





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Overview

Product name Anti-Claudin 4 antibody [EPRR17575]

Description Rabbit monoclonal [EPRR17575] to Claudin 4

Host species Rabbit

Specificity The mouse recommendation is based on the WB results. We do not guarantee IHC-P for mouse.

Tested applications Suitable for: IHC-P, WB, IP Species reactivity Reacts with: Mouse. Human

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

Positive control WB: LNCaP, PC-3, SW480, MCF7, SK-OV-3, NIH:OVCAR-3, 4T1. IHC-P: Human colon,

endometrium, liver, cholangiocarcinoma and breast cancer tissues. IP: SW480 whole cell lysate.

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, 0.05% BSA

Purity Protein A purified

Clonality Monoclonal Clone number EPRR17575

Isotype IgG

Applications

The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab210796 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
IHC-P	**** <u>(2)</u>	1/4000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		1/1000. Detects a band of approximately 18 kDa (predicted molecular weight: 22 kDa).
IP		1/30.

Target

Function Plays a major role in tight junction-specific obliteration of the intercellular space.

Involvement in disease Note=CLDN4 is located in the Williams-Beuren syndrome (WBS) critical region. WBS results

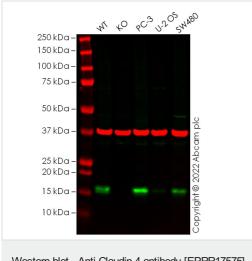
from a hemizygous deletion of several genes on chromosome 7q11.23, thought to arise as a consequence of unequal crossing over between highly homologous low-copy repeat sequences

flanking the deleted region.

Sequence similarities Belongs to the claudin family.

Cellular localization Cell junction > tight junction. Cell membrane.

Images



Western blot - Anti-Claudin 4 antibody [EPRR17575] (ab210796)

All lanes : Anti-Claudin 4 antibody [EPRR17575] (ab210796) at 1/1000 dilution

Lane 1: Wild-type MCF7 cell lysate

Lane 2: CLDN4 knockout MCF7 cell lysate

Lane 3 : PC-3 cell lysate

Lane 4 : U-2 OS cell lysate

Lane 5 : SW480 cell lysate

Lysates/proteins at 20 µg per lane.

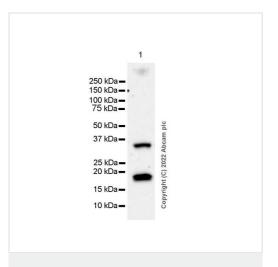
Secondary

All lanes : Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 22 kDa Observed band size: 17 kDa

False colour image of Western blot: Anti-Claudin 4 antibody [EPRR17575] staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab210796 was shown to bind specifically to Claudin 4. A band was observed at 17 kDa in wild-type MCF7 cell lysates with no signal observed at this size in CLDN4 knockout cell line ab274946 (knockout cell lysate ab275004). To generate this image, wild-type and CLDN4 knockout MCF7 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution 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 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.



Western blot - Anti-Claudin 4 antibody [EPRR17575] (ab210796)

Anti-Claudin 4 antibody [EPRR17575] (ab210796) at 1/1000 dilution + 4T1 (mouse mammary gland carcinoma epithelial cell), whole cell lysate at 20 µg

Secondary

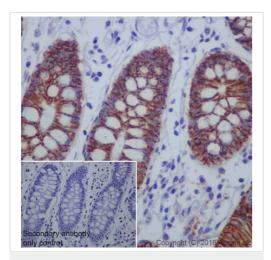
Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 22 kDa **Observed band size:** 18 kDa

Exposure time: 3 minutes

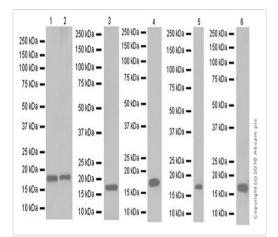
Blocking and diluting buffer and concentration: 5% NFDM/TBST

The identity of the higher MW band at approximately 35 kDa is unknown.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Claudin 4 antibody

[EPRR17575] (ab210796)



Western blot - Anti-Claudin 4 antibody [EPRR17575] (ab210796)

Immunohistochemical analysis of paraffin-embedded human colon tissue labeling Claudin 4 with ab210796 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

Membrane and staining on epithelial cells of human colon is observed.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

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

Lanes 1-2: Anti-Claudin 4 antibody [EPRR17575] (ab210796) at 1/2000 dilution

Lanes 3-6: Anti-Claudin 4 antibody [EPRR17575] (ab210796) at 1/1000 dilution

Lane 1: LNCaP (Human prostate cancer cell line) whole cell lysate

Lane 2 : PC-3 (Human prostate adenocarcinoma cell line) whole cell lysate

Lane 3 : SW480 (Human colorectal adenocarcinoma cell line) whole cell lysate

Lane 4 : MCF7 (Human breast adenocarcinoma cell line) whole cell lysate

Lane 5: SK-OV-3 (Human ovarian cancer cell line) whole cell lysate

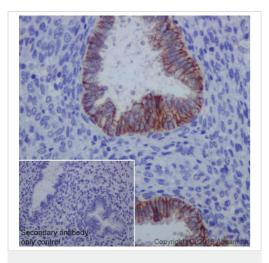
Lane 6 : NIH:OVCAR-3 (Human ovary adenocarcinoma cell line) whole cell lysate

Lysates/proteins at 10 µg per lane.

Secondary

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

Predicted band size: 22 kDa Observed band size: 18 kDa Exposure time: Lane 1/2/3/6: 1 minute; Lane 4: 30 seconds; Lane 5: 3 minutes.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Claudin 4 antibody
[EPRR17575] (ab210796)

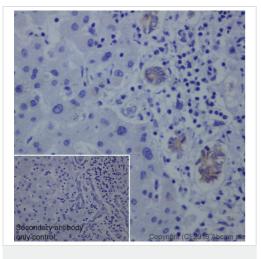
Immunohistochemical analysis of paraffin-embedded human endometrium tissue labeling Claudin 4 with ab210796 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

Membrane staining on epithelial cells of human endometrium is observed.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

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



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Claudin 4 antibody
[EPRR17575] (ab210796)

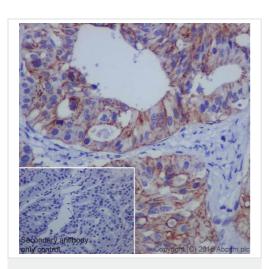
Immunohistochemical analysis of paraffin-embedded human liver tissue labeling Claudin 4 with ab210796 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

Weak membrane staining on biliary epithelium of human liver is observed.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

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



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Claudin 4 antibody
[EPRR17575] (ab210796)

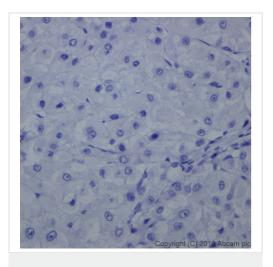
Immunohistochemical analysis of paraffin-embedded human cholangiocarcinoma tissue labeling Claudin 4 with ab210796 at 1/4000 dilution, followed by Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

Membrane staining on human cholangiocarcinoma is observed.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

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



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Claudin 4 antibody

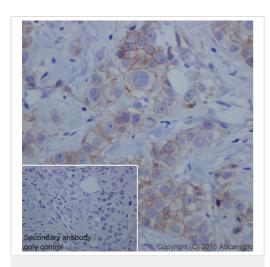
[EPRR17575] (ab210796)

Immunohistochemical analysis of paraffin-embedded human hepatocellular carcinoma tissue labeling Claudin 4 with ab210796 at 1/4000 dilution, followed by Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

Negative staining on human hepatocellular carcinoma.

Counter stained with Hematoxylin.

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



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Claudin 4 antibody
[EPRR17575] (ab210796)

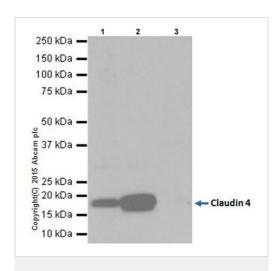
Immunohistochemical analysis of paraffin-embedded human breast cancer tissue labeling Claudin 4 with ab210796 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

Membrane staining on epithelial cells of human breast cancer is observed.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

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



Immunoprecipitation - Anti-Claudin 4 antibody [EPRR17575] (ab210796)

Claudin 4 was immunoprecipitated from 1mg of SW480 (Human colorectal adenocarcinoma cell line) whole cell lysate with ab210796 at 1/30 dilution.

Western blot was performed from the immunoprecipitate using ab210796 at 1/1000 dilution.

VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>), was used for detection at 1/10000 dilution.

Lane 1: SW480 whole cell lysate 10µg (Input).

Lane 2: ab210796 IP in SW480 whole cell lysate.

Lane 3: Rabbit monoclonal $\lg G$ ($\underline{ab172730}$) instead of ab210796 in SW480 whole cell lysate.

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

Exposure time: 30 seconds.



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