# abcam

## Product datasheet

## Anti-Claudin 1 antibody [EPRR18871] ab211737





## ★★★★★ 7 Abreviews 25 References 14 Images

#### Overview

**Product name** Anti-Claudin 1 antibody [EPRR18871]

**Description** Rabbit monoclonal [EPRR18871] to Claudin 1

**Host species** Rabbit

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

Species reactivity Reacts with: Human, Recombinant fragment

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

Positive control WB: HEK-293, A431 and HepG2 whole cell lysates; human fetal liver and fetal kidney lysates;

> human Claudin 1 full length recombinant protein. IHC-P: Human skin, cervix, colon cancer and lung squamous cell cancer tissues. ICC/IF: A431 and HepG2 cells. Flow Cyt (intra): HepG2 cells. IP:

HepG2 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** 

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 EPRR18871

**Isotype** IgG

#### **Applications**

#### The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab211737 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 (Intra)		1/60.
ICC/IF		1/1000.
IP		1/40.
IHC-P	<b>★★★★</b> (3)	1/250. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		1/2000. Detects a band of approximately 18 kDa (predicted molecular weight: 22 kDa).

#### **Target**

#### **Function**

Claudins function as major constituents of the tight junction complexes that regulate the permeability of epithelia. While some claudin family members play essential roles in the formation of impermeable barriers, others mediate the permeability to ions and small molecules. Often, several claudin family members are coexpressed and interact with each other, and this determines the overall permeability. CLDN1 is required to prevent the paracellular diffusion of small molecules through tight junctions in the epidermis and is required for the normal barrier function of the skin. Required for normal water homeostasis and to prevent excessive water loss through the skin, probably via an indirect effect on the expression levels of other proteins, since CLDN1 itself seems to be dispensable for water barrier formation in keratinocyte tight junctions (PubMed:23407391).

(Microbial infection) Acts as a receptor for hepatitis C virus in hepatocytes (PubMed:17325668). Acts as a receptor for dengue virus (PubMed:24074594).

Tissue specificity

Strongly expressed in liver and kidney. Expressed in heart, brain, spleen, lung and testis.

Involvement in disease

lchthyosis-sclerosing cholangitis neonatal syndrome

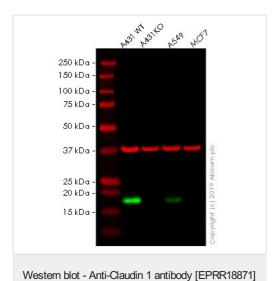
Sequence similarities

Belongs to the claudin family.

**Cellular localization** 

Cell junction, tight junction. Cell membrane.

#### **Images**



(ab211737)

**All lanes :** Anti-Claudin 1 antibody [EPRR18871] (ab211737) at 1/2000 dilution

**Lane 1 :** Wild-type A-431 (Human epidermoid carcinoma cell line) whole cell lysate

Lane 2: CLDN1 knockout A-431 (Human epidermoid carcinoma cell line) whole cell lysate

Lane 3 : A549 (Human lung carcinoma cell line) whole cell lysate

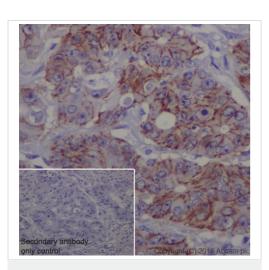
Lane 4 : MCF7 (Human breast adenocarcinoma cell line) whole
cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 22 kDa

**Lanes 1 - 4:** Merged signal (red and green). Green - ab211737 observed at 18 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab211737 was shown to specifically react with in wild-type A431 WT cells as signal was lost in A431 CLDN1 knockout cells. Wild-type and A431 CLDN1 knockout samples were subjected to SDS-PAGE. The membrane was blocked with 3pc Milk. Ab211737 and ab8245 (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 1/2000 dilution and 1/20000 dilution respectively. Blots were developed 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 1/20000 dilution for 1 hour at room temperature before imaging.

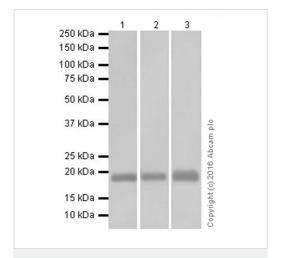


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Claudin 1 antibody
[EPRR18871] (ab211737)

Immunohistochemical analysis of paraffin-embedded human colon cancer tissue labeling Claudin 1 with ab211737 at 1/250 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Membrane staining on human colon 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.



Western blot - Anti-Claudin 1 antibody [EPRR18871] (ab211737)

**All lanes :** Anti-Claudin 1 antibody [EPRR18871] (ab211737) at 1/2000 dilution

**Lane 1 :** HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate

Lane 2 : A431 (Human epidermoid carcinoma cell line) whole cell lysate

**Lane 3 :** HepG2 (Human liver hepatocellular carcinoma cell line) whole cell lysate

Lysates/proteins at 10 µg per lane.

#### Secondary

**All lanes :** Goat Anti-Rabbit  $\lg G \ H\&L \ (HRP) \ (\underline{ab97051})$  at 1/100000 dilution

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

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1/2: 15 seconds; Lane 3: 5 seconds.

The expression profile/ molecular weight observed is consistent with what has been described in the literature PMID: 26601075.

**All lanes :** Anti-Claudin 1 antibody [EPRR18871] (ab211737) at 1/2000 dilution

Lane 1 : Human fetal liver lysate

Lane 2 : Human fetal kidney lysate

Lysates/proteins at 10 µg per lane.

## **Secondary**

**All lanes :** Goat Anti-Rabbit IgG Peroxidase Conjugate, specific to the non-reduced form of IgG at 1/10000 dilution

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

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

The expression profile/ molecular weight observed is consistent with what has been described in the literature PMID: 26601075.

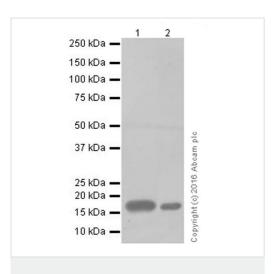
Anti-Claudin 1 antibody [EPRR18871] (ab211737) at 1/5000 dilution + Human Claudin 1 full length recombinant protein at 0.01 µg

#### **Secondary**

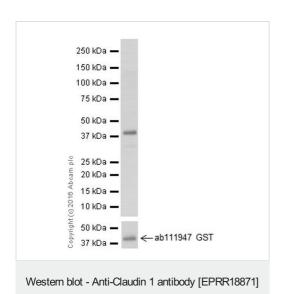
Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

**Predicted band size:** 22 kDa **Observed band size:** 45 kDa

Exposure time: 1 second



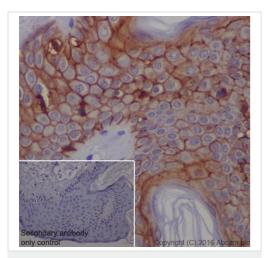
Western blot - Anti-Claudin 1 antibody [EPRR18871] (ab211737)



(ab211737)

Blocking/Dilution buffer: 5% NFDM/TBST.

Human Claudin 1 full length recombinant protein contains aa1-211 with His-Tag® and GST-tag. This recombinant was made in house.

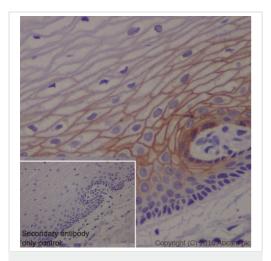


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Claudin 1 antibody
[EPRR18871] (ab211737)

Immunohistochemical analysis of paraffin-embedded human skin tissue labeling Claudin 1 with ab211737 at 1/250 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Membrane staining on human skin squamous cells 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.

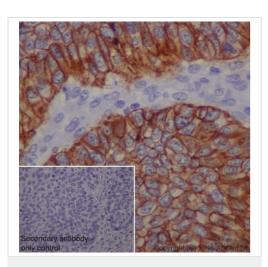


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Claudin 1 antibody
[EPRR18871] (ab211737)

Immunohistochemical analysis of paraffin-embedded human cervix tissue labeling Claudin 1 with ab211737 at 1/250 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Membrane staining on human cervix 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.

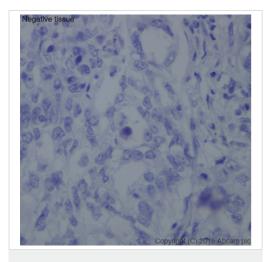


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Claudin 1 antibody
[EPRR18871] (ab211737)

Immunohistochemical analysis of paraffin-embedded human lung squamous cell cancer tissue labeling Claudin 1 with ab211737 at 1/250 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Membrane staining on human lung squamous cell 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.



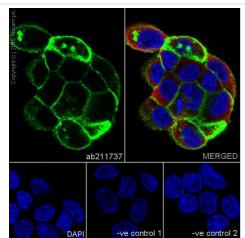
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Claudin 1 antibody
[EPRR18871] (ab211737)

Immunohistochemical analysis of paraffin-embedded human lung adenocarcinoma tissue labeling Claudin 1 with ab211737 at 1/250 dilution, followed by Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

**Negative control**: Negative staining on human lung adenocarcinoma, which is consistent with the literature (PMID: 17585317).

Counter stained with Hematoxylin.

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



Immunocytochemistry/ Immunofluorescence - Anti-Claudin 1 antibody [EPRR18871] (ab211737)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HepG2 (Human liver hepatocellular carcinoma cell line) cells labeling Claudin 1 with ab211737 at 1/1000 dilution, followed by Goat Anti-Rabbit lgG H&L (Alexa Fluor<sup>®</sup> 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing membranous staining on HepG2 cell line. The nuclear counterstain is DAPI (blue).

(ab150077) secondary antibody at 1/1000 dilution.

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized A431 (Human epidermoid carcinoma cell line) cells labeling Claudin 1 with ab211737 at 1/1000 dilution,

Confocal image showing membrane staining on A431 cell line. The

Tubulin is detected with Anti-alpha Tubulin mouse MAb (ab7291) at 1/1000 dilution and Goat Anti-Mouse IgG H&L (Alexa Fluor<sup>®</sup> 594)

-ve control 1: ab211737 at 1/1000 dilution followed by Goat Anti-

-ve control 2: Anti-alpha Tubulin mouse MAb (ab7291) at 1/1000 dilution followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488)

Mouse IgG H&L (Alexa Fluor® 594) (ab150120) secondary

followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green).

(ab150120) secondary antibody at 1/1000 dilution (red).

nuclear counterstain is DAPI (blue).

The negative controls are as follows:-

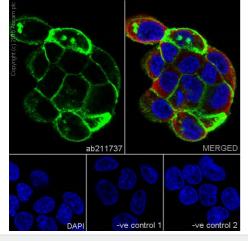
antibody at 1/1000 dilution.

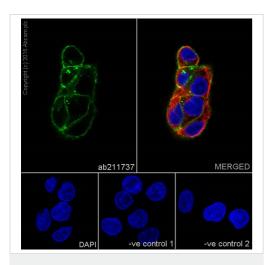
Tubulin is detected with Anti-alpha Tubulin mouse MAb (ab7291) at 1/1000 dilution and Goat Anti-Mouse IgG H&L (Alexa Fluor<sup>®</sup> 594) (ab150120) secondary antibody at 1/1000 dilution (red).

The negative controls are as follows:-

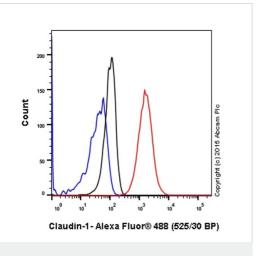
-ve control 1: ab211737 at 1/1000 dilution followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (ab150120) secondary antibody at 1/1000 dilution.

-ve control 2: Anti-alpha Tubulin mouse MAb (ab7291) at 1/1000 dilution followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution.



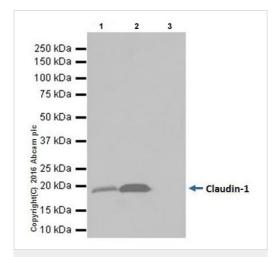


Immunocytochemistry/ Immunofluorescence - Anti-Claudin 1 antibody [EPRR18871] (ab211737)



Flow Cytometry (Intracellular) - Anti-Claudin 1 antibody [EPRR18871] (ab211737)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed HepG2 (Human liver hepatocellular carcinoma cell line) cells labeling Claudin 1with ab211737 at 1/60 dilution (red) compared with a rabbit monoclonal lgG isotype control (ab172730; black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody; blue). Goat Anti-Rabbit lgG (Alexa Fluor® 488) at 1/2000 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-Claudin 1 antibody [EPRR18871] (ab211737)

Claudin 1 was immunoprecipitated from 0.35 mg of HepG2 (Human liver hepatocellular carcinoma cell line) whole cell lysate with ab211737 at 1/40 dilution.

Western blot was performed from the immunoprecipitate using ab211737 at 1/2000 dilution.

VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>), was used for detection at 1/10,000 dilution

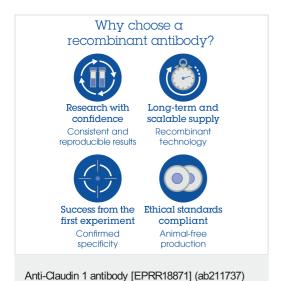
Lane 1: HepG2 whole cell lysate 10µg (Input).

Lane 2: ab211737 IP in HepG2 whole cell lysate.

Lane 3: Rabbit monoclonal IgG (<u>ab172730</u>) instead of ab211737 in HepG2 whole cell lysate.

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

Exposure time: 3 seconds.



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