Anti-Claudin 1 Antibody [PS01-43] - BSA and Azide free

HA750567

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse
Applications:	WB, IHC-P, IF-Tissue, IF-Cell
Molecular Wt:	Predicted band size: 23 kDa
Clone number:	PS01-43

Description: 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. Acts as a co-receptor for hepatitis C virus (HCV) in hepatocytes. Associates with CD81 and the CLDN1-CD81 receptor complex is essential for HCV entry into host cell.

Immunogen: Recombinant full length protein within human Claudin 1.

Positive control: A431 cell lysates, A431, HepG2, human tonsil tissue, human skin tissue, mouse skin tissue.

Subcellular location: Cell junction, Cell membrane, Membrane, Tight junction

Database links: SwissProt: O95832 Human | O88551 Mouse

Recommended Dilutions:

WB	1:1,000
IHC-P	1:1,000
IF-Tissue	1:200-1:500
IF-Cell	1:100

Storage Buffer: 1*PBS (pH7.4).

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

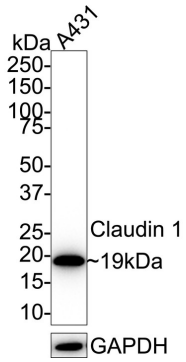

Fig1: Western blot analysis of Claudin 1 on A431 cell lysates with Rabbit anti-Claudin 1 antibody (HA750567) at 1/1,000 dilution.

Lysates/proteins at 10 µg/Lane.

Predicted band size: 23 kDa
Observed band size: 19 kDa

Exposure time: 25 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA750567) at 1/1,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

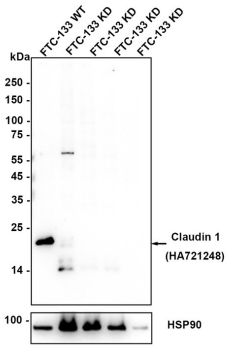

Fig2: Western blot analysis of Claudin 1 on different lysates with Rabbit anti-Claudin 1 antibody (HA750567) at 1/1,000 dilution.

Lane 1: Wild-type FTC-133 whole cell lysate (10 µg).
Lane 2/3/4: Claudin 1 knockdown FTC-133 whole cell lysate (10 µg).

Predicted band size: 23 kDa
Observed band size: 23 kDa

Exposure time: 3 minutes; ECL: K1802;4-20% SDS-PAGE gel.

HA750567 was shown to specifically react with Claudin 1 in wild-type FTC-133 cells. Weakened bands were observed when Claudin 1 knockdown samples were tested. Wild-type and Claudin 1 knockdown samples were subjected to SDS-PAGE. Proteins were transferred to a PVDF membrane and blocked with 5% NFDM in TBST for 1 hour at room temperature. The primary antibody (HA750567) at 1/1,000 dilution were used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

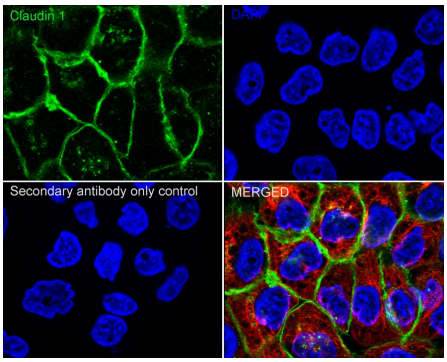

Fig3: Immunocytochemistry analysis of A431 cells labeling Claudin 1 with Rabbit anti-Claudin 1 antibody (HA750567) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Claudin 1 antibody (HA750567) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

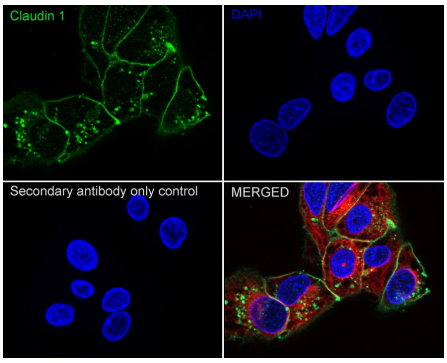

Fig4: Immunocytochemistry analysis of HepG2 cells labeling Claudin 1 with Rabbit anti-Claudin 1 antibody (HA750567) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Claudin 1 antibody (HA750567) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

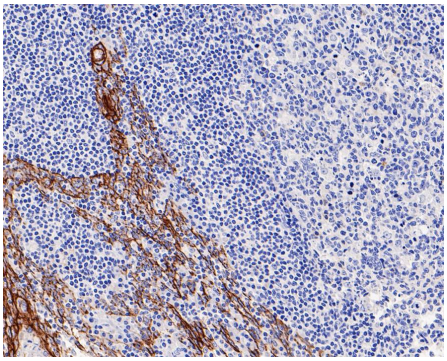

Fig5: Immunohistochemical analysis of paraffin-embedded human tonsil tissue with Rabbit anti-Claudin 1 antibody (HA750567) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750567) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

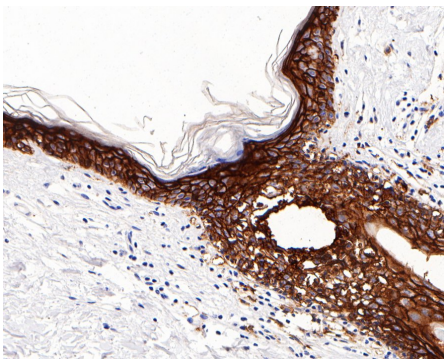

Fig6: Immunohistochemical analysis of paraffin-embedded human skin tissue with Rabbit anti-Claudin 1 antibody (HA750567) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750567) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

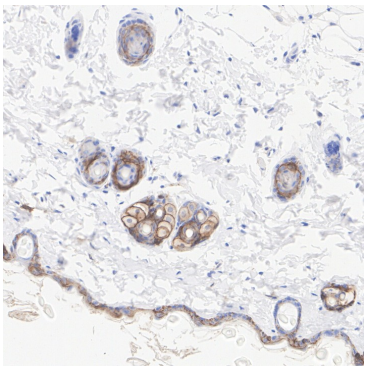

Fig7: Immunohistochemical analysis of paraffin-embedded mouse skin tissue with Rabbit anti-Claudin 1 antibody (HA750567) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750567) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

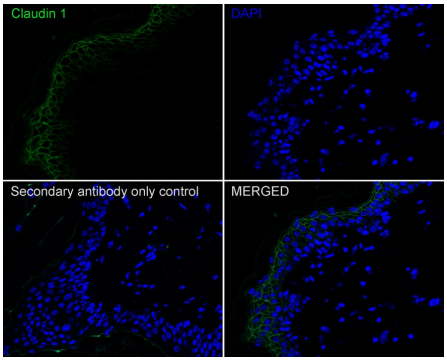

Fig8: Immunofluorescence analysis of paraffin-embedded human skin tissue labeling Claudin 1 with Rabbit anti-Claudin 1 antibody (HA750567) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (HA750567, green) at 1/200 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

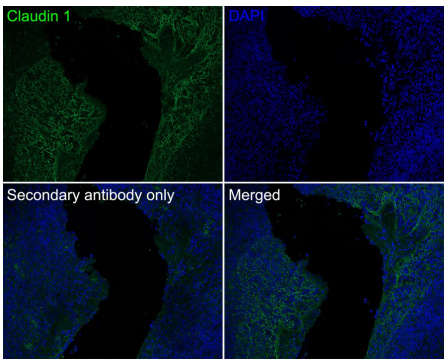

Fig9: Immunofluorescence analysis of paraffin-embedded human tonsil tissue labeling Claudin 1 with Rabbit anti-Claudin 1 antibody (HA750567) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (HA750567, green) at 1/200 dilution overnight at 4 ℃, washed with PBS.

Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

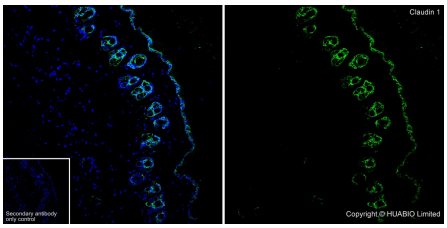

Fig10: Application: Immunofluorescence (IF-tissue)

Species: Mouse
Tissue: Skin
Sample: Paraffin-embedded section

Antigen retrieval: Heat-mediated, Tris-EDTA buffer (pH 9.0), 20 minutes at 95℃.

Wash buffer: 1× PBS
Endogenous peroxidase blocking: 3% H O , 10 minutes.
Blocking: 1% BSA + 10% normal goat serum, 10 minutes at room temperature.
Primary antibody: HA750567, 1/500, overnight at 4℃.
Secondary antibody: Goat Anti-Rabbit IgG (iFluor™ 488, HA1121), 1.5 hours at room temperature.

Background References

1. Kirschner N., Rosenthal R., Furuse M., Moll I., Fromm M., Brandner J.M. Contribution of tight junction proteins to ion, macromolecule, and water barrier in keratinocytes. J. Invest. Dermatol. 133:1161-1169 (2013)
2. Harris H.J., Davis C., Mullins J.G., Hu K., Goodall M., Farquhar M.J., Mee C.J., McCaffrey K., Young S., Drummer H., Balfe P., McKeating J.A. Claudin association with CD81 defines hepatitis C virus entry. J. Biol. Chem. 285:21092-21102 (2010)