Anti-Occludin Antibody

R1510-33



Product Type: Rabbit polyclonal IgG, primary antibodies

Species reactivity: Human, Mouse, Rat

Applications: WB, IF-Cell, IHC-P, FC

Molecular Wt: Predicted band size: 59 kDa

Description: This gene encodes an integral membrane protein that is required for cytokine-induced

regulation of the tight junction paracellular permeability barrier. Mutations in this gene are thought to be a cause of band-like calcification with simplified gyration and polymicrogyria (BLC-PMG), an autosomal recessive neurologic disorder that is also known as pseudo-TORCH syndrome. Alternative splicing results in multiple transcript variants. A related

pseudogene is present 1.5 Mb downstream on the q arm of chromosome 5.

Immunogen: Synthetic peptide within human Occludin aa 6-45 conjugated to Keyhole Limpet

Haemocyanin (KLH).

Positive control: Mouse kidney tissue lysate, human kidney tissue lysate, Caco-2, human lung tissue, mouse

kidney tissue, HepG2.

Subcellular location: Cell membrane.

Database links: SwissProt: Q16625 Human | Q61146 Mouse | Q6P6T5 Rat

Recommended Dilutions:

WB 1:500-2,000 IF-Cell 1:2,000 IHC-P 1:50-1:200 FC 1:50-1:100

Storage Buffer: 1*PBS (pH7.4), 0.2% BSA, 50% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at $+4^{\circ}$ C after thawing. Aliquot store at -20° C. Avoid repeated freeze / thaw cycles.

Purity: Immunogen affinity purified.

Hangzhou Huaan Biotechnology Co., Ltd.



Service mail:support@huabio.cn



Images

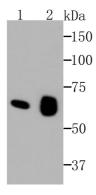
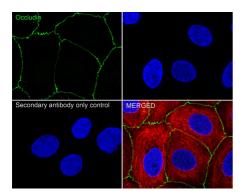


Fig1: Western blot analysis of Occludin on different lysates. Proteins were transferred to a PVDF membrane and blocked with 5% BSA in PBS for 1 hour at room temperature. The primary antibody (R1510-33, 1/500) was used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:200,000 dilution was used for 1 hour at room temperature.

Positive control:

Lane 1: Mouse kidney tissue lysate Lane 2: Human kidney tissue lysate

Fig2: Immunocytochemistry analysis of Caco-2 cells labeling Occludin with Rabbit anti-Occludin antibody (R1510-33) at 1/2,000 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-Occludin antibody (R1510-33) at 1/2,000 dilution in 1% BSA in PBST overnight at 4 $^{\circ}$ C. 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 $+4^{\circ}$ C. Goat Anti-Mouse IgG H&L (iFluor ** 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

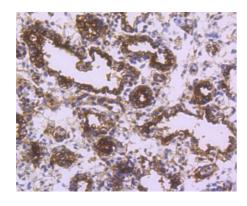


Fig3: Immunohistochemical analysis of paraffin-embedded human lung tissue using anti-Occludin antibody. The section was pretreated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 30 minutes at room temperature, washed with ddH $_2$ O and PBS, and then probed with the primary antibody (R1510-33, 1/50) for 30 minutes 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.

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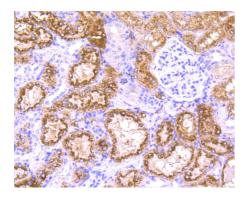


Fig4: Immunohistochemical analysis of paraffin-embedded mouse kidney tissue using anti-Occludin antibody. The section was pretreated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 30 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (R1510-33, 1/50) for 30 minutes 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.

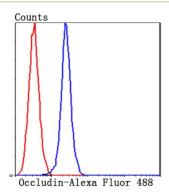


Fig5: Flow cytometric analysis of Occludin was done on HepG2 cells. The cells were fixed, permeabilized and stained with the primary antibody (R1510-33, 1/50) (blue). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor®488 conjugate-Goat anti-Rabbit IgG Secondary antibody at 1/1000 dilution for 30 minutes.Unlabelled sample was used as a control (cells without incubation with primary antibody; red).

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

- 1. Okai K et al. A novel occludin-targeting monoclonal antibody prevents hepatitis C virus infection in vitro. Oncotarget. 2018 Mar 30;9(24):16588-16598.
- 2. Ogawa M et al. Effect of interleukin-1β on occludin mRNA expression in the duodenal and colonic mucosa of dogs with inflammatory bowel disease. J Vet Intern Med. 2018 Mar 23.