Anti-Occludin Antibody [JJ091-08] - BSA and Azide free

HA752011

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat, Cynomolgus monkey, Pig
Applications:	WB, IP, FC, IHC-P, IHC-Fr, IF-Tissue, mIHC
Molecular Wt:	Predicted band size: 59 kDa
Clone number:	JJ091-08

Description: Occludin is an integral membrane protein closely associated with the tight junctions of epithelial and endothelial cells. Occludin is a tetraspan integral membrane protein in epithelial and endothelial tight junction (TJ) structures that can contain two extracellular loops. The protein exists in a variety of phosphorylated forms. Phosphorylation is involved in regulating both the localization and the function of occludin. Expression of occludin is up-regulated by poly-unsaturated fatty acids, increasing transendothelial cell resistance and reducing cellular permeability to large molecules. The level of occludin varies greatly depending on tissue; in brain tissue, occludin is highly expressed at cell-cell contact sites. Non-neural tissues show lower expression and discontinuous distribution. Up-regulation of epithelial occludin may play a role in enhancing paracellular permeability and be related to the damage to the tight junction.

Immunogen: Synthetic peptide within N-terminal human Occludin.

Positive control: Human colon tissue, mouse colon tissue, rat colon tissue, Caco-2 cell lysate, Mouse smooth muscle tissue lysate.

Subcellular location: Cell membrane, tight junction.

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

Recommended Dilutions:

WB	1:1,000-1:2,000
FC	1:1,000
IP	Use at an assay dependent concentration.
IHC-P	1:100-1:500
IHC-Fr	1:500
IF-Tissue	1:500
mIHC	1:50

Storage Buffer: 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

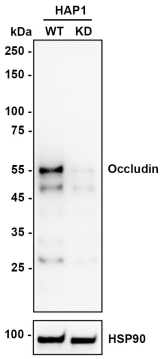

Fig1: Western blot analysis of Occludin on different lysates with Rabbit anti-Occludin antibody (HA752011) at 1/2,000 dilution.

Lane 1: HAP1-parental cell lysate
Lane 2: HAP1-Occludin KD cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 59 kDa
Observed band size: 59 kDa

Exposure time: 180 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 (HA752011) at 1/2,000 dilution was used in K1803 at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

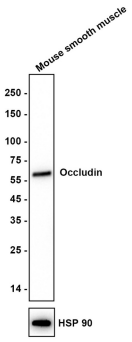

Fig2: Western blot analysis of Occludin on Mouse smooth muscle tissue lysate with Rabbit anti-Occludin antibody (ET1701-76) at 1/1,000 dilution.

Lysates/proteins at 10 µg/Lane.
Exposure time: 25 seconds; ECL: K1801

Blocking: 5% NFDM/TBST, 1 hour at room temperature
Primary antibody: ET1701-76, 1/1,000 in 5% NFDM/TBST, overnight at 4 ℃
Secondary antibody: Goat anti-Rabbit IgG-HRP (HA1001), 1/50,000 in 5% NFDM/TBST, 1 hour at room temperature

Predicted band size: 59.1 kDa
Observed band size: 59 kDa

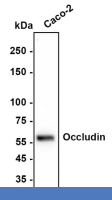

Fig3: Western blot analysis of Occludin on Caco-2 cell lysate with Rabbit anti-Occludin antibody (ET1701-76) at 1/1,000 dilution.

Lysates/proteins at 10 µg/Lane.
Exposure time: 120 seconds; ECL: K1801

Blocking: 5% NFDM/TBST, 1 hour at room temperature

Observed band size: 59 kDa

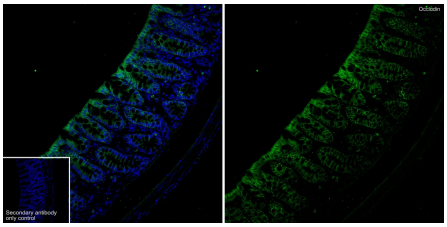

Fig4: Application: IHC-Fr

Species: Mouse

Site: Colon

Sample: Frozen section

Antibody concentration: 1:500

Antigen retrieval: Not required

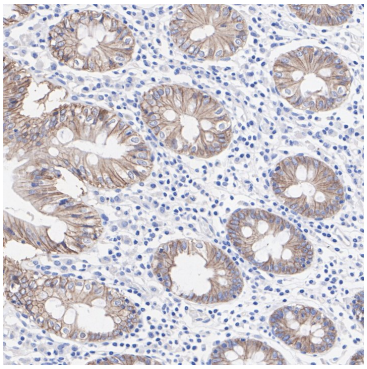

Fig5: Immunohistochemical analysis of paraffin-embedded human colon tissue with Rabbit anti-Occludin antibody (HA752011) at 1/100 dilution.

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 (HA752011) at 1/100 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.

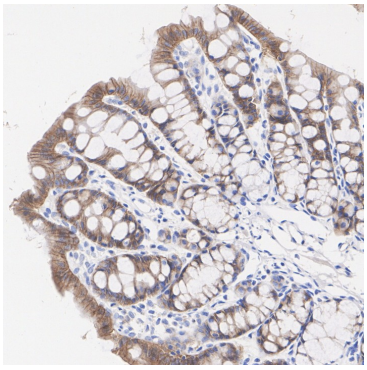

Fig6: Immunohistochemical analysis of paraffin-embedded mouse colon tissue with Rabbit anti-Occludin antibody (HA752011) at 1/500 dilution.

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 (HA752011) at 1/500 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.

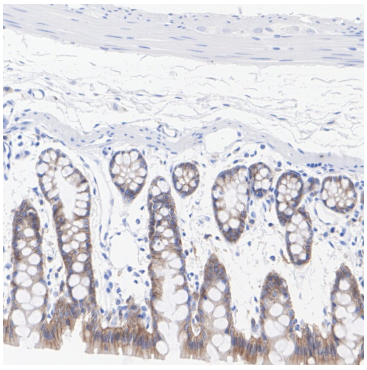

Fig7: Immunohistochemical analysis of paraffin-embedded rat colon tissue with Rabbit anti-Occludin antibody (HA752011) at 1/100 dilution.

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 (HA752011) at 1/100 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.

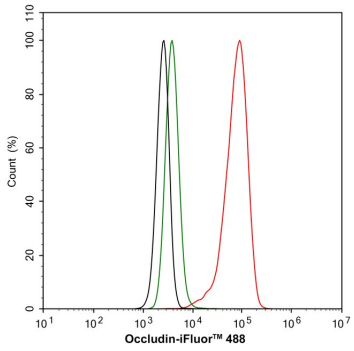

Fig8: Flow cytometric analysis of Caco-2 cells labeling Occludin.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA752011, 1μg/mL) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

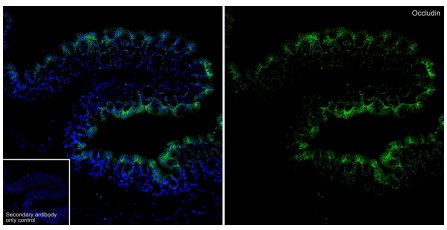

Fig9: Application: IF-Tissue

Species: Mouse

Site: colon

Sample: Paraffin-embedded section

Antibody concentration: 1/500

Background References

1. Cao S et al. Hydrogen sulfide attenuates brain edema in early brain injury after subarachnoid hemorrhage in rats: Possible involvement of MMP-9 induced blood-brain barrier disruption and AQP4 expression. Neurosci Lett 621:88-97 (2016).
2. Zhang C et al. Catalpol downregulates vascular endothelial-cadherin expression and induces vascular hyperpermeability. Mol Med Rep 13:373-8 (2016).