Anti-Occludin Antibody [JJ091-08]

ET1701-76

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IP, FC, IF-Cell
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: MCF-7 cell lysate, Mouse smooth muscle tissue lysate, Caco-2.

Subcellular location: Cell membrane, tight junction.

Database links: SwissProt: Q16625 Human | Q61146 Mouse

Recommended Dilutions:

WB	1:1,000
FC	1:1,000
IP	Use at an assay dependent concentration.
IF-Cell	1:500

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

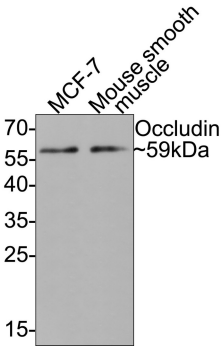

Fig1: Western blot analysis of Occludin on different lysates with Rabbit anti-Occludin antibody (ET1701-76) at 1/1,000 dilution.

Lane 1: MCF-7 cell lysate (10 µg/Lane)
Lane 2: Mouse smooth muscle tissue lysate (20 µg/Lane)

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

Exposure time: 2 minutes;

12% 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 (ET1701-76) 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 1:300,000 dilution was used for 1 hour at room temperature.

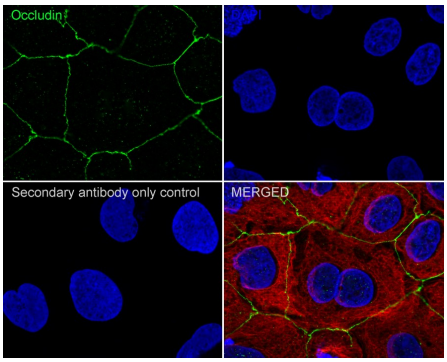

Fig2: Immunocytochemistry analysis of Caco-2 cells labeling Occludin with Rabbit anti-Occludin antibody (ET1701-76) at 1/500 dilution.

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 (ET1701-76) at 1/500 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.

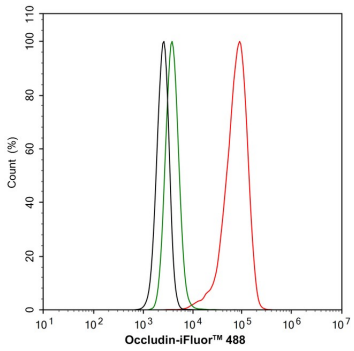

Fig3: Flow cytometric analysis of Caco-2 cells labeling Occludin.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1701-76, 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).

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

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).