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Occludin Monoclonal Antibody (OC-3F10), HRP

Catalog Number 33-1520

Product data sheet

Details	
Size	100 µg
Host/Isotope	Mouse / IgG1, kappa
Class	Monoclonal
Type	Antibody
Clone	OC-3F10
Immunogen	GST fusion protein consisting of the C-terminal region (~150aa) of human occludin.
Conjugate	HRP
Form	Liquid
Concentration	1 mg/mL
Purification	Protein A
Storage buffer	PBS, pH 7.4, with 4mg/mL BSA, 40% glycerol
Contains	0.19% Kathon™ CG
Storage Conditions	4° C

Species Reactivity	
Species reactivity	Dog, Human, Mouse, Rat
Published species	Dog, Rat, Human, Mouse, Not Applicable

Tested Applications	Dilution *
ELISA (ELISA)	0.1-1.0 µg/mL
Western Blot (WB)	2 µg/mL

Published Applications	
Western Blot (WB)	See 3 publications below
ELISA (ELISA)	See 2 publications below
Immunocytochemistry (ICC/IF)	See 1 publications below

* Suggested working dilutions are given as a guide only. It is recommended that the user titrate the product for use in their own experiment using appropriate negative and positive controls.

Background/Target Information

Occludin is a 65 kDa protein that can exist in a variety of phosphorylated forms, ranging up to approximately 82 kDa. Occludin is thought to be involved in regulating both the localization and the function of occludin. Polyunsaturated fatty acids are known to up-regulate occludin expression, increasing the transendothelial cell resistance and reducing the cellular permeability to large molecules. The level of occludin varies greatly depending on tissue; in brain tissue, occludin is highly and continuously expressed at cell-cell contact sites, whereas non-neural tissues show lower expression and discontinuous distribution. Overall structural features of the occludin protein are highly conserved in all the species examined. Under-expression of tight junction proteins, including occludin, is a key molecular abnormality responsible for the increased permeability of tumor endothelial tight junctions, which contributes to brain tumor edemas.

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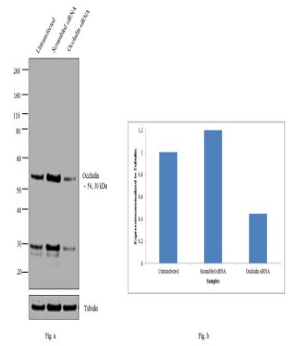
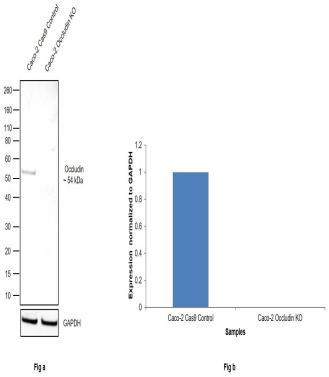
Product Images For Occludin Monoclonal Antibody (OC-3F10), HRP



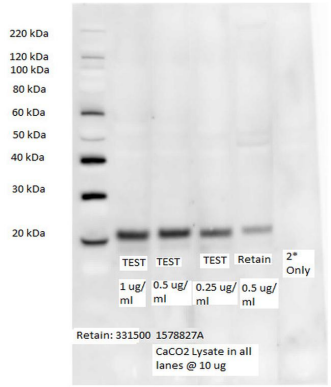
Occludin Antibody (33-1520)

Antibody specificity was demonstrated by siRNA mediated knockdown of target protein. Caco-2 cells were transfected with Occludin siRNA and reduction of signal was observed in Western Blot using Occludin Monoclonal Antibody (Product # 33-1520). {KD}



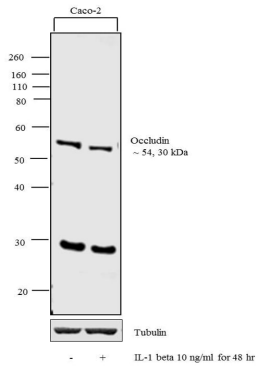
Occludin Antibody (33-1520) in WB

Knockout of Occludin was achieved by CRISPR-Cas9 genome editing using LentiArray™ Lentiviral sgRNA (Product # A32042, Assay ID CRISPR777079_LV) and LentiArray Cas9 Lentivirus (Product # A32064). Western blot analysis of Occludin was performed by loading 30 µg of Caco-2 Cas9 (Lane 1) andCaco-2 Occludin KO (Lane 2) membrane enriched extracts. The samples were electrophoresed using NuPAGE™ Novex™ 4-12% Bis-Tris Protein Gel (Product # NP0322BOX). Resolved proteins were then transferred onto a nitrocellulose membrane (Product # IB23001) by iBlot® 2 Dry Blotting System (Product # IB21001). The blot was probed with Anti-Occludin Monoclonal Antibody-HRP (OC-3F10) (Product # 33-1520, 1:500 dilution) using the iBright FL 1000 (Product # A32752). Chemiluminescent detection was performed using Novex® ECL Chemiluminescent Substrate Reagent Kit (Product # WP20005). Loss of signal upon CRISPR mediated knockout (KO) using the LentiArray™ CRISPR product line confirms that antibody is specific to Occludin.



Occludin Antibody (33-1520) in WB

Western blot analysis of Occludin was performed by loading 10 µg of CaCO2 lysate per well onto a 4-12% Bis-Tris polyacrylamide gel. Proteins were transferred to a nitrocellulose membrane and blocked with 5% BSA/TBST for at least 1 hour. The membrane was probed with an Occludin purified monoclonal antibody (Product # 33-1500) at a dilution of 1 µg/mL, 0.5 µg/mL, and 0.25 µg/mL for 1 hour at room temperature on a rocking platform and washed in TBS-0.1% Tween-20. Goat Anti-Mouse IGG (H+L) HRP Conjugated secondary antibody (Product # 31430) was used at a concentration of 1:20,000 and incubated for 30 minutes on a rocking platform. Super Signal West Dura substrate (Product # 34076) was incubated for 5 minutes on a rocking platform. Detection was performed using the GBox. The 5th isoform of Occludin was detected at ~23 kDa.



Occludin Antibody (33-1520)

Altered expression of target protein upon cell treatment demonstrates antibody specificity. Western blot analysis of Occludin using with Occludin Monoclonal Antibody (Product # 33-1520) shows a decrease in the protein level upon IL-1 beta treatment in Caco-2 cell line. {TM}

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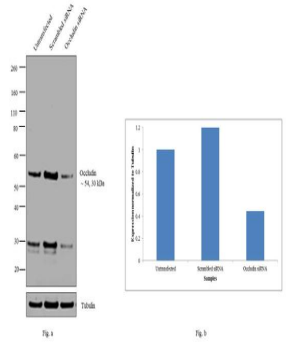
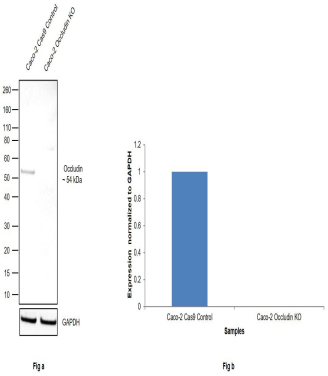


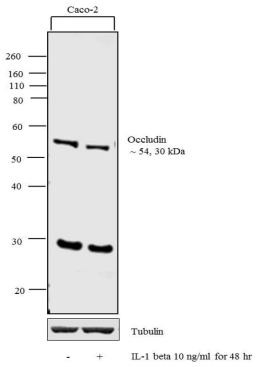
Occludin Antibody (33-1520) in WB

Knockdown of Occludin was achieved by transfecting Caco-2 with Occludin specific siRNAs (Silencer® select Product # s9812, s9814). Western blot analysis (Fig. a) was performed using whole cell extracts from the Occludin knockdown cells (lane 3), non-specific scrambled siRNA transfected cells (lane 2) and untransfected cells (lane 1). The blots were probed with Occludin Monoclonal Antibody (OC-3F10), HRP (Product # 33-1520, 2µg/ml). Densitometric analysis of this western blot is shown in histogram (Fig. b). Decrease in signal upon siRNA mediated knock down confirms that antibody is specific to Occludin.



Occludin Antibody (33-1520)

Antibody specificity was demonstrated by CRISPR-Cas9 mediated knockout of target protein. A loss of signal was observed for target protein inOccludin KO cell line compared to control cell line using Anti-Occludin Monoclonal Antibody (OC-3F10), HRP (Product # 33-1520). {KO}



Occludin Antibody (33-1520) in WB

Western blot analysis was performed on whole cell extracts (30 µg lysate) of Caco-2 (Lane 1) and Caco-2 treated with IL 1 beta (10 ng/ml for 48 hr) (Lane 2). The blot was probed with Anti-Occludin Monoclonal Antibody (OC-3F10), HRP (Product # 33-1520, 2 µg/ml). A 54, 30 kDa bands corresponding to Occludin was observed in the cell line tested and decreased upon treatment.

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PubMed References For Occludin Monoclonal Antibody (OC-3F10), HRP

3 Western Blot References

Species / Dilution	Summary
Rat / Not Cited	The Journal of biological chemistry (2000; 275: 7125) **"Role of megalin (gp330) in transcytosis of thyroglobulin by thyroid cells. A novel function in the control of thyroid hormone release."** Author(s):Marinò M,Zheng G,Chiovato L,Pinchera A,Brown D,Andrews D,McCluskey RT PubMed Article URL:http://dx.doi.org/10.1074/jbc.275.10.7125
Human / 1:1000	33-1520 was used in Western Blotting to indicate that HDAC inhibitors suppress the proliferation, migration and invasiveness of HNSCC by downregulating the p63-mediated tight junction molecules JAM-A and claudin-1, and inducing p63 or p21-mediated growth arrest. Oncology reports (2021; 45:) **"HDAC inhibitors suppress the proliferation, migration and invasiveness of human head and neck squamous cell carcinoma cells via p63mediated tight junction molecules and p21mediated growth arrest."** Author(s):Kakiuchi A,Kakuki T,Ohwada K,Kurose M,Kondoh A,Obata K,Nomura K,Miyata R,Kaneko Y,Konno T,Kohno T,Himi T,Takano KI,Kojima T PubMed Article URL:http://dx.doi.org/10.3892/or.2021.7997
Human / Not Cited	Journal of agricultural and food chemistry (2012; 60: 4628) **"Differential effects of flavonoids on barrier integrity in human intestinal Caco-2 cells."** Author(s):Noda S,Tanabe S,Suzuki T PubMed Article URL:http://dx.doi.org/10.1021/jf300382h

2 ELISA References

Species / Dilution	Summary
Human / Not Cited	33-1520 was used in an ELISA assay to study the effects of fingolimod on circulating tight-junction protein levels as well as on peripheral blood mononuclear cells migration. Scientific reports (2018; 8:) **"Fingolimod reduces circulating tight-junction protein levels and in vitro peripheral blood mononuclear cells migration in multiple sclerosis patients."** Author(s):Annunziata P,Cioni C,Masi G,Tassi M,Marotta G,Severi S PubMed Article URL:http://dx.doi.org/10.1038/s41598-018-33672-9
Mouse / Not Cited	33-1520 was used in Enzyme-linked immunosorbent assay to show that brain pericytes, the mural cells of the capillary walls, differentially modulate endothelial cell phenotype in an apoE isoform-dependent manner. Arteriosclerosis, thrombosis, and vascular biology (2020; 40: 128) **"ApoE (Apolipoprotein E) in Brain Pericytes Regulates Endothelial Function in an Isoform-Dependent Manner by Modulating Basement Membrane Components."** Author(s):Yamazaki Y,Shinohara M,Yamazaki A,Ren Y,Asmann YW,Kanekiyo T,Bu G PubMed Article URL:http://dx.doi.org/10.1161/ATVBAHA.119.313169

1 Immunocytochemistry References

Species / Dilution	Summary
Human / Not Cited Dog / Not Cited	The Biochemical journal (2011; 437: 289) **"Protein kinase C phosphorylates occludin and promotes assembly of epithelial tight junctions."** Author(s):Jain S,Suzuki T,Seth A,Samak G,Rao R PubMed Article URL:http://dx.doi.org/10.1042/BJ20110587

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