abcam

Product datasheet

Anti-Occludin antibody [EPR20992] - BSA and Azide free ab224526

KO VALIDATED | Recombinant | RabMAb

10 Images

Overview

Product name	Anti-Occludin antibody [EPR20992] - BSA and Azide free
Description	Rabbit monoclonal [EPR20992] to Occludin - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** IHC-P, WB, Flow Cyt, ICC/IF, IP
Species reactivity	**Reacts with:** Mouse, Rat, Dog, Human
Immunogen	Recombinant fragment within Human Occludin aa 350 to the C-terminus. The exact sequence is proprietary. Database link: Q16625
Positive control	IHC-P: Human colon tissue.
General notes	Ab224526 is the carrier-free version of ab216327. This format is designed for use in antibody labeling, including fluorochromes, metal isotopes, oligonucleotides, enzymes. Our carrier-free formats are supplied in a buffer free of BSA, sodium azide and glycerol for higher conjugation efficiency. Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. ab224526 is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm. *Maxpar® is a trademark of Fluidigm Canada Inc.* This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information see here. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents. Reproducibility is key to advancing scientific discovery and accelerating scientists' next

breakthrough.

Abcam is leading the way with our range of recombinant antibodies, knockout-validated antibodies and knockout cell lines, all of which support improved reproducibility.

We are also planning to innovate the way in which we present recommended applications and species on our product datasheets, so that only applications & species that have been tested in our own labs, our suppliers or by selected trusted collaborators are covered by our Abpromise™ guarantee.

In preparation for this, we have started to update the applications & species that this product is Abpromise guaranteed for.

We are also updating the applications & species that this product has been "predicted to work with," however this information is not covered by our Abpromise guarantee.

Applications & species from publications and Abreviews that have not been tested in our own labs or in those of our suppliers are not covered by the Abpromise guarantee.

Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, as well as customer reviews and Q&As.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR20992
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab224526** in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		Use at an assay dependent concentration. Detects a band of approximately 65, 53, 25, 23 kDa (predicted molecular weight: 59 kDa).
Flow Cyt		Use at an assay dependent concentration.

Application	Abreviews	Notes
ICC/IF		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.

Target

Function
May play a role in the formation and regulation of the tight junction (TJ) paracellular permeability barrier. It is able to induce adhesion when expressed in cells lacking tight junctions.

Tissue specificity
Localized at tight junctions of both epithelial and endothelial cells. Highly expressed in kidney. Not detected in testis.

Involvement in disease
Defects in OCLN are the cause of band-like calcification with simplified gyration and polymicrogyria (BLCPMG) [MIM:251290]; also known as pseudo-TORCH syndrome. BLCPMG is a neurologic disorder with characteristic clinical and neuroradiologic features that mimic intrauterine TORCH infection in the absence of evidence of infection. Affected individuals have congenital microcephaly, intracranial calcifications, and severe developmental delay.

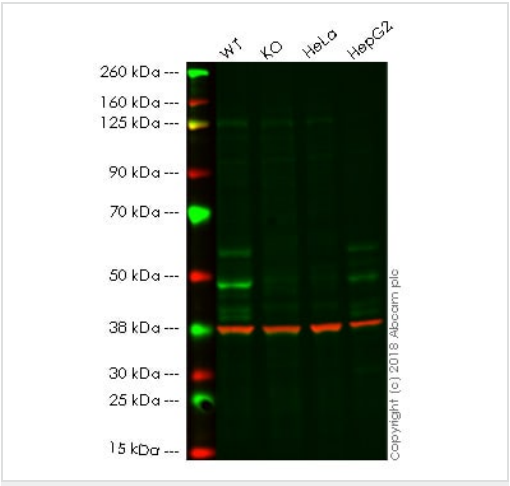
Sequence similarities
Belongs to the ELL/occludin family.
Contains 1 MARVEL domain.

Domain
The C-terminal is cytoplasmic and is important for interaction with ZO-1. Sufficient for the tight junction localization. Involved in the regulation of the permeability barrier function of the tight junction (By similarity). The first extracellular loop participates in an adhesive interaction.

Post-translational modifications
Phosphorylated upon DNA damage, probably by ATM or ATR. Dephosphorylated by PTPRJ. The tyrosine phosphorylation on Tyr-398 and Tyr-402 reduces its ability to interact with TJP1.

Cellular localization
Membrane. Cell junction > tight junction.

Images



Western blot - Anti-Occludin antibody [EPR20992] - BSA and Azide free (ab224526)

All lanes : Anti-Occludin antibody [EPR20992] (ab216327) at 1/1000 dilution

Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : OCLN (Occludin) knockout HAP1 whole cell lysate
Lane 3 : HeLa whole cell lysate (Low Occludin expression)
Lane 4 : HepG2 whole cell lysate lysate (High Occludin expression)

Lysates/proteins at 40 µg per lane.

Predicted band size: 59 kDa

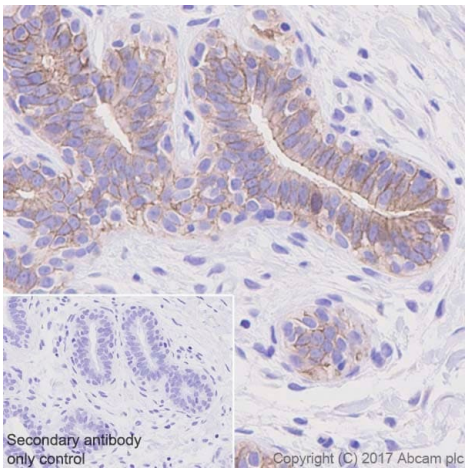
Lanes 1 - 4: Merged signal (red and green). Green - ab216327

observed at 59 kDa. Red - loading control, ab9484, observed at 37 kDa.

ab216327 was shown to recognize Occludin in wild-type HAP1 cells as signal was lost at the expected MW in OCLN (Occludin) knockout cells. Additional cross-reactive bands were observed in the wild-type and knockout cells. Wild-type and OCLN (Occludin) knockout samples were subjected to SDS-PAGE. Ab216327 and ab9484 (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed ab216773 and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed ab216776 secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.

Occludin expression in HeLa is expected to be negative.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab216327).



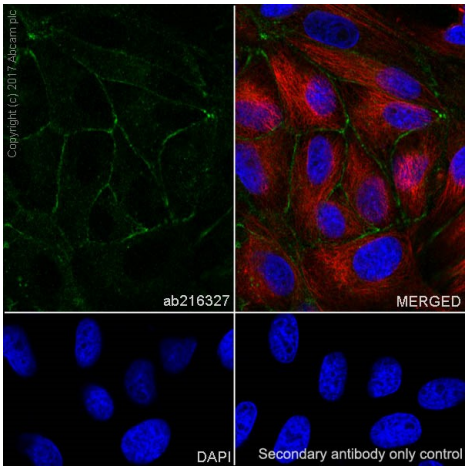
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Occludin antibody [EPR20992] - BSA and Azide free (ab224526)

Immunohistochemical analysis of paraffin-embedded human breast tissue labeling Occludin with ab216327 at 1/200 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) ready to use. Membranous staining on human breast is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) ready to use.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab216327).

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



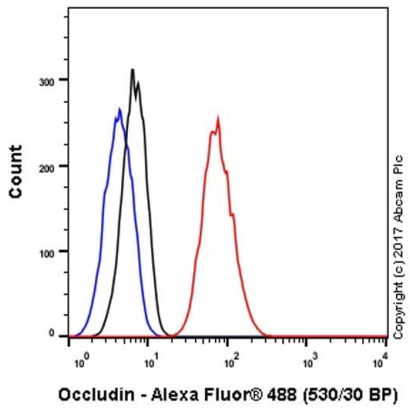
Immunocytochemistry/ Immunofluorescence - Anti-Occludin antibody [EPR20992] - BSA and Azide free (ab224526)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized MDCK (canine kidney cell line) cells labeling Occludin with ab216327 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing membrane staining on MDCK (NBL-2) cell line.

The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (ab195889) at 1/200 dilution (red).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution.

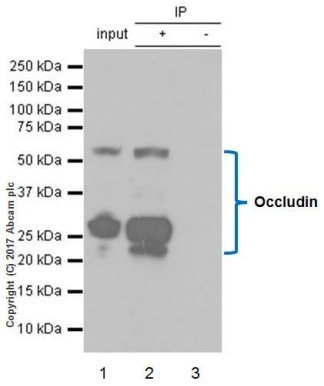
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab216327).



Flow Cytometry - Anti-Occludin antibody [EPR20992] - BSA and Azide free (ab224526)

Flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol-permeabilized Caco-2 (human colorectal adenocarcinoma cell line) cell line labeling Occludin with ab216327 at 1/60 dilution (red) compared with a Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730) (black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) at 1/2000 dilution was used as the secondary antibody.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab216327).



Immunoprecipitation - Anti-Occludin antibody [EPR20992] - BSA and Azide free (ab224526)

Occludin was immunoprecipitated from 0.35 mg of Caco-2 (human colorectal adenocarcinoma cell line) whole cell lysate with ab216327 at 1/40 dilution. Western blot was performed from the immunoprecipitate using ab216327 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/10000 dilution.

Lane 1: Caco-2 whole cell lysate 10 µg (Input).

Lane 2: ab216327 IP in Caco-2 whole cell lysate.

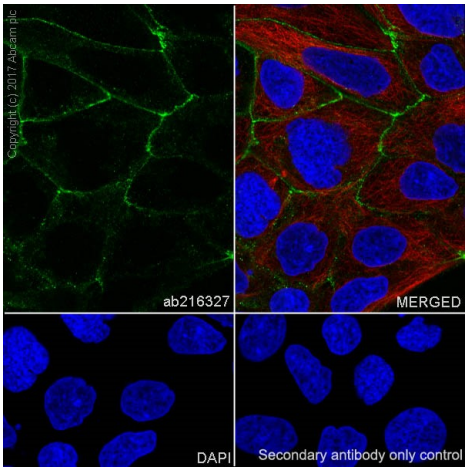
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab216327 in Caco-2 whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 1 second.

The molecular weight observed is consistent with what has been described in the literature (PMID: 18647175, PMID: 19821483).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab216327).



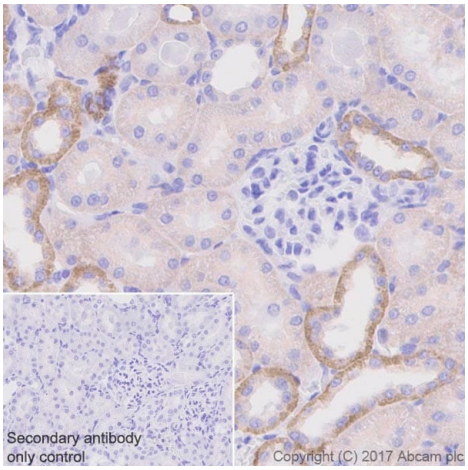
Immunocytochemistry/ Immunofluorescence - Anti-Occludin antibody [EPR20992] - BSA and Azide free (ab224526)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized Caco-2 (human colorectal adenocarcinoma cell line) cells labeling Occludin with ab216327 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing membrane staining on Caco-2 cell line.

The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (ab195889) at 1/200 dilution (red).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab216327).



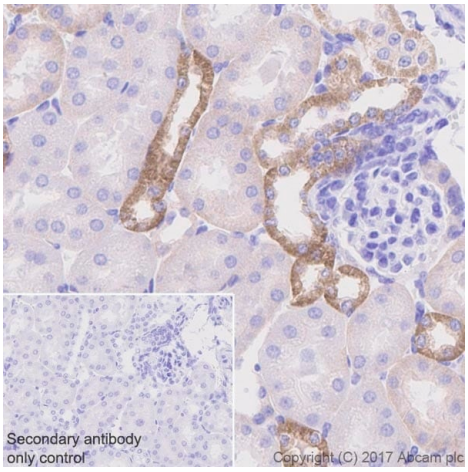
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Occludin antibody [EPR20992] - BSA and Azide free (ab224526)

Immunohistochemical analysis of paraffin-embedded rat kidney tissue labeling Occludin with ab216327 at 1/200 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) ready to use. Cytoplasmic staining on distal tubules of rat kidney is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) ready to use.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab216327).

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



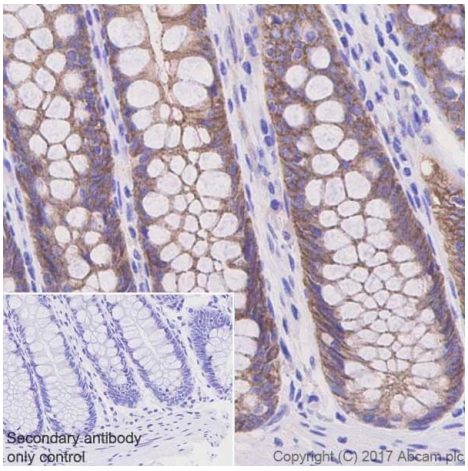
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Occludin antibody [EPR20992] - BSA and Azide free (ab224526)

Immunohistochemical analysis of paraffin-embedded mouse kidney tissue labeling Occludin with ab216327 at 1/200 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) ready to use. Cytoplasmic staining on distal tubules of mouse kidney is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) ready to use.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab216327).

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



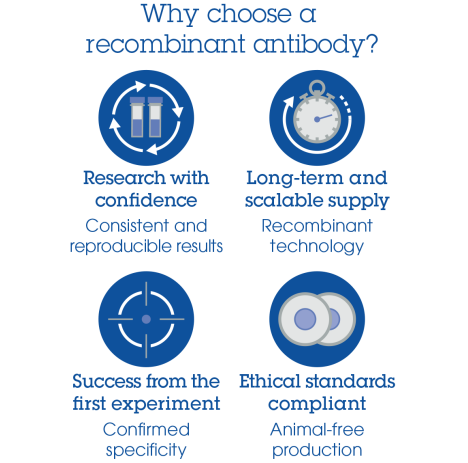
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Occludin antibody [EPR20992] - BSA and Azide free (ab224526)

Immunohistochemical analysis of paraffin-embedded human colon tissue labeling Occludin with ab216327 at 1/200 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) ready to use. Membranous staining on human colon is observed (PMID: 24268521). Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) ready to use.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab216327).

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Anti-Occludin antibody [EPR20992] - BSA and Azide free (ab224526)

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