

Product datasheet

Anti-Occludin antibody [EPR20992] ab216327

KO VALIDATED Recombinant RabMAB

★★★★☆ 7 Abreviews 165 References 17 Images

Overview

Product name	Anti-Occludin antibody [EPR20992]
Description	Rabbit monoclonal [EPR20992] to Occludin
Host species	Rabbit
Specificity	This antibody is not suitable for ICC in mouse species or for any testing rat intestinal tissues.
Tested applications	Suitable for: WB, IHC-P, ICC/IF, IP, Flow Cyt (Intra)
Species reactivity	Reacts with: Mouse, Rat, Dog, Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Human colon lysate; MDCK, PC-3, HEK-293 and Caco-2 whole cell lysates; Rat brain and lung lysate; Mouse colon, brain, lung and uterus lysates; bEnd.3 whole cell lysate. IHC-P: Human colon and breast tissues; human thyroid carcinoma; Mouse and rat kidney tissues. ICC/IF: Caco-2 and MDCK cells. Flow Cyt (intra): Caco-2 cells. IP: Caco-2 whole cell lysate.
General notes	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none">- High batch-to-batch consistency and reproducibility- Improved sensitivity and specificity- Long-term security of supply- Animal-free production <p>For more information see here.</p> <p>Our RabMAB[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAB[®] patents.</p>

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 0.05% BSA, 40% Glycerol, PBS
Purity	Protein A purified

Clonality	Monoclonal
Clone number	EPR20992
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab216327 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
WB	★★★★☆ (3)	1/1000. Detects a band of approximately 65, 53, 25, 23 kDa (predicted molecular weight: 59 kDa). Please upload more of the lysate or use lower dilution of ab216327 when testing bEnd.3 and mouse intestinal tissues. ab216327 is not suitable for testing rat intestinal tissues.
IHC-P	★★★★★ (3)	1/200. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		1/100. This antibody is not suitable for ICC in mouse species.
IP		1/40.
Flow Cyt (Intra)		1/60.

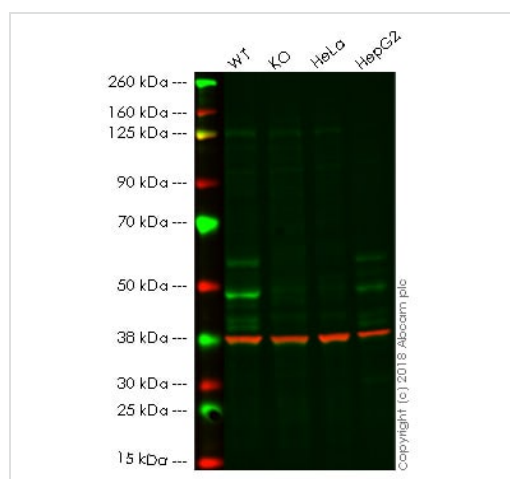
Target

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.

Tissue Microarray (TMA) data for ab216327					
Normal tissue samples			Malignant tissue samples		
Human cardiac muscle	x	Human placenta	✓	Clear cell carcinoma of human kidney	x
Human cerebrum	* (endothelial cells ✓)	Human skeletal muscle	x	Human bladder cancer	✓
Human colon	✓	Human skin	✓	Human breast carcinoma	✓
Human endometrium	✓	Human spleen	* (endothelial cells ✓)	Human cervical carcinoma	✓
Human kidney	✓	Human stomach	✓	Human colon carcinoma	✓
Human liver	x	Human testis	✓	Human endometrial carcinoma	✓
Human lung	✓	Human thyroid	✓	Human gastric adenocarcinoma	✓
Human mammary gland	✓	Human tonsil	* (endothelial cells ✓)	Human glioma	x
Human pancreas	✓			Human hepatocellular carcinoma	✓
				Human lung carcinoma	✓
				Human ovarian carcinoma	✓
				Human pancreatic carcinoma	✓
				Human prostatic hyperplasia	x
				Human thyroid carcinoma	✓

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Occludin antibody [EPR20992] (ab216327)

Tissue Microarrays stained for Anti-Occludin antibody [EPR20992] using ab216327 in immunohistochemical analysis. This table provides a detailed overview of positive (tick mark) and negative (cross mark) staining per sample type tested. The sections were incubated with ab216327 at 4°C overnight used at 1:2000 dilution (1.05 µg/ml) followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB secondary antibody ([ab209101](#)). Counterstain was Hematoxylin. Heat mediated antigen retrieval was performed using [ab93684](#) (Tris/EDTA buffer, pH 9.0).



Western blot - Anti-Occludin antibody [EPR20992] (ab216327)

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

Lane 1 : Wild-type HAP1 whole cell lysate at 40 µg

Lane 2 : OCLN (Occludin) knockout HAP1 whole cell lysate at 40 µg

Lane 3 : HeLa whole cell lysate (Low Occludin expression) at 20 µg

Lane 4 : HepG2 whole cell lysate lysate (High Occludin expression) at 20 µg

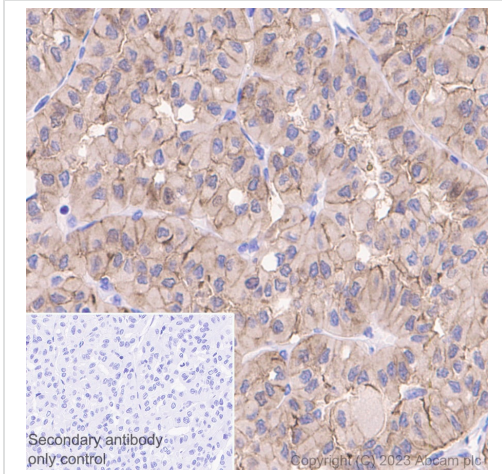
Predicted band size: 59 kDa

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.

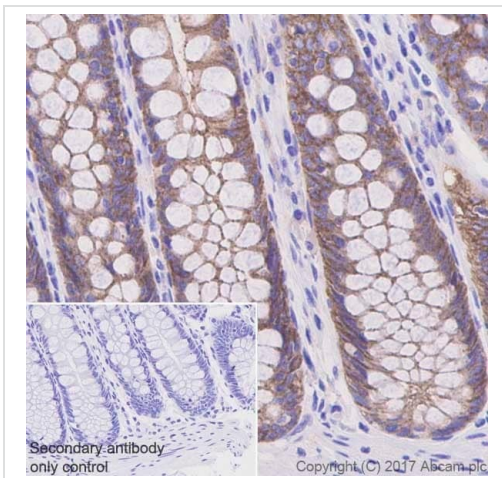


Immunohistochemical analysis of paraffin-embedded human thyroid carcinoma labeling Occludin with ab216327 at 1/200 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP polymer) (**ab214880**), ready to use. Counter stained with Hematoxylin. Antigen retrieval was heat mediated antigen retrieval was performed using **ab93684** (Tris/EDTA buffer, pH 9.0).

Secondary antibody only control: **ab209101** (Rabbit specific IHC polymer detection kit HRP/DAB).

Positive staining on human thyroid carcinoma. The section was incubated with ab216327 at 4°C overnight.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Occludin antibody [EPR20992] (ab216327)

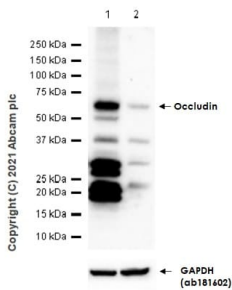


Immunohistochemical analysis of paraffin-embedded human colon tissue labeling Occludin with ab216327 at 1/200 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) ready to use. Membranous staining on human colon is observed (PMID: 24268521). Counter stained with Hematoxylin.

Secondary antibody only control: **ab209101** (Rabbit specific IHC polymer detection kit HRP/DAB).

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] (ab216327)



Western blot - Anti-Occludin antibody [EPR20992] (ab216327)

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

Lane 1 : Caco-2 (Human colorectal adenocarcinoma epithelial cell) whole cell lysate

Lane 2 : bEnd.3 (Mouse brain endothelioma) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

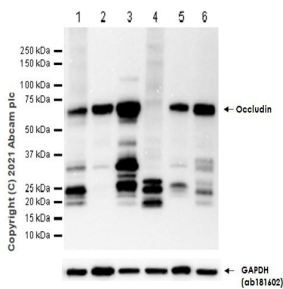
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/10000 dilution

Predicted band size: 59 kDa

Observed band size: 65 kDa

Exposure time: 60 seconds

Blocking/Dilution buffer: 5% NFDm/TBST.



Western blot - Anti-Occludin antibody [EPR20992] (ab216327)

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

Lane 1 : Mouse colon tissue lysate

Lane 2 : Mouse brain tissue lysate

Lane 3 : Mouse lung tissue lysate

Lane 4 : Rat colon tissue lysate

Lane 5 : Rat brain tissue lysate

Lane 6 : Rat lung tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary

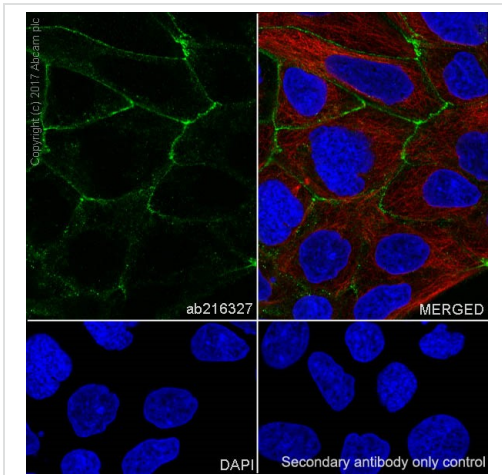
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/10000 dilution

Predicted band size: 59 kDa

Observed band size: 65 kDa

Exposure time: 100 seconds

Blocking/Dilution buffer: 5% NFDm/TBST.

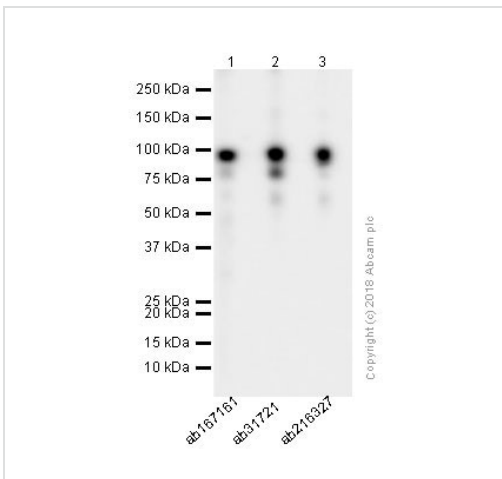


Immunocytochemistry/ Immunofluorescence - Anti-Occludin antibody [EPR20992] (ab216327)

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.



Western blot - Anti-Occludin antibody [EPR20992] (ab216327)

Lane 1 : Anti-Occludin antibody [EPR8208] ([ab167161](#)) at 1/5000 dilution

Lane 2 : Anti-Occludin antibody ([ab31721](#)) at 1/1000 dilution

Lane 3 : Anti-Occludin antibody [EPR20992] (ab216327) at 1/5000 dilution

All lanes : Recombinant Human Occludin protein ([ab114189](#))

Lysates/proteins at 0.025 µg per lane.

Secondary

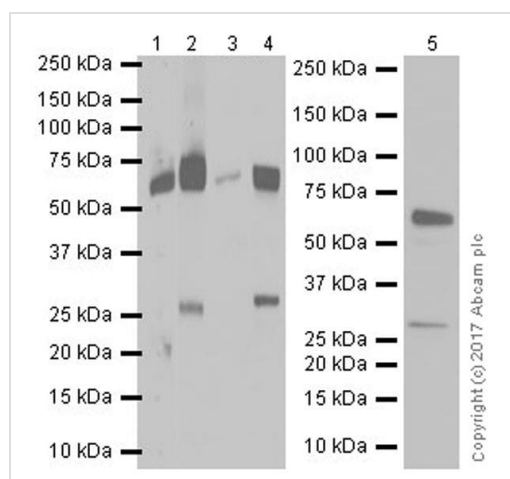
All lanes : Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/20000 dilution

Predicted band size: 59 kDa

Observed band size: 85 kDa

Blocking and diluting buffer: 5% NFDm/TBST

Exposure time: 5.5 seconds



Western blot - Anti-Occludin antibody [EPR20992]
(ab216327)

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

Lane 1 : Human colon lysate at 20 µg

Lane 2 : MDCK (canine kidney cell line) cell lysate at 20 µg

Lane 3 : PC-3 (human prostate adenocarcinoma cell line) cell lysate at 20 µg

Lane 4 : HEK-293 (human epithelial cell line from embryonic kidney) cell lysate at 20 µg

Lane 5 : Caco-2 (human colorectal adenocarcinoma cell line) cell lysate at 10 µg

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/100000 dilution

Developed using the ECL technique.

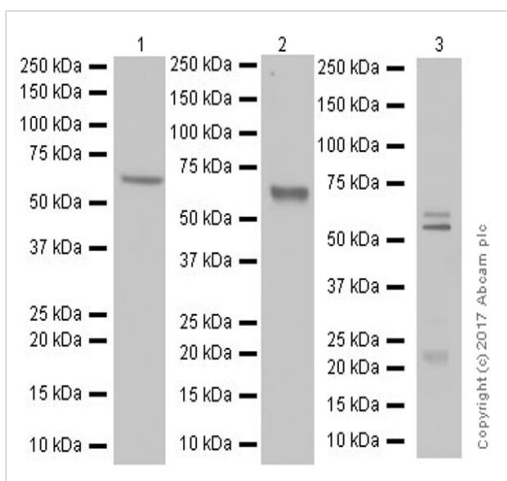
Predicted band size: 59 kDa

Observed band size: 25,53,65 kDa

Exposure time : Lanes 1-4: 3 minutes; lane 5: 1 minute.

Blocking/Dilution buffer: 5% NFDm/TBST.

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



Western blot - Anti-Occludin antibody [EPR20992] (ab216327)

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

Lane 1 : Rat brain lysate

Lane 2 : Mouse brain lysate

Lane 3 : Mouse uterus lysate

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Developed using the ECL technique.

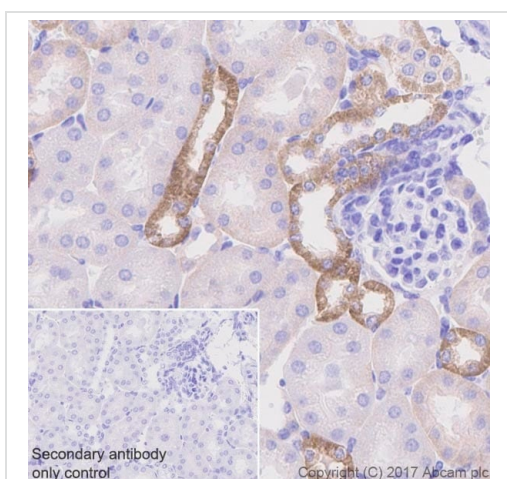
Predicted band size: 59 kDa

Observed band size: 23,53,65 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFD/MTBST.

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

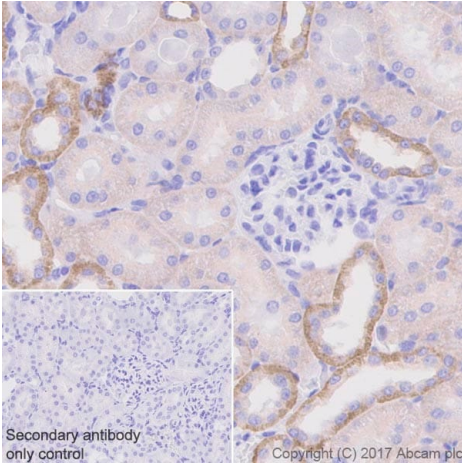


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Occludin antibody [EPR20992] (ab216327)

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: **ab209101** (Rabbit specific IHC polymer detection kit HRP/DAB).

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

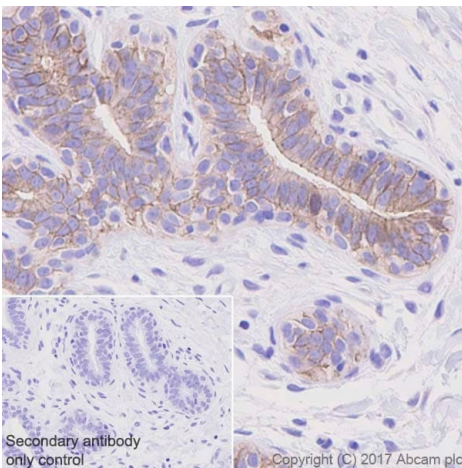


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Occludin antibody [EPR20992] (ab216327)

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: **ab209101** (Rabbit specific IHC polymer detection kit HRP/DAB).

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

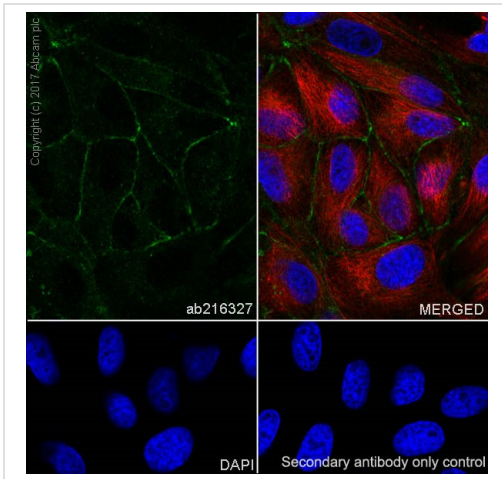


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Occludin antibody [EPR20992] (ab216327)

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: **ab209101** (Rabbit specific IHC polymer detection kit HRP/DAB).

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

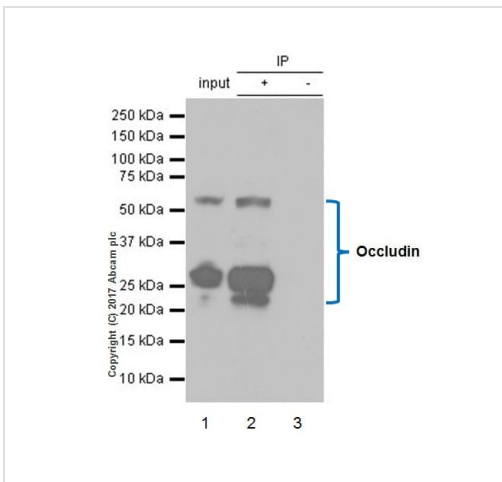


Immunocytochemistry/ Immunofluorescence - Anti-Occludin antibody [EPR20992] (ab216327)

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.



Immunoprecipitation - Anti-Occludin antibody [EPR20992] (ab216327)

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 1/10,000 dilution

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

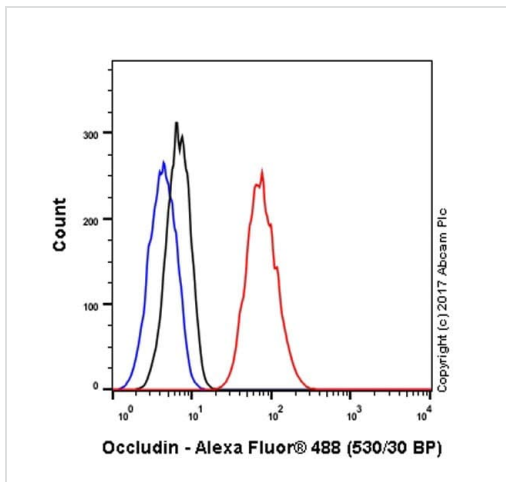
Lane 2: ab216327 IP in Caco-2 whole cell lysate.

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

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

Exposure time: 1 second.

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



Flow Cytometry (Intracellular) - Anti-Occludin antibody [EPR20992] (ab216327)

Intracellular 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.

Why choose a recombinant antibody?

 <p>Research with confidence Consistent and reproducible results</p>	 <p>Long-term and scalable supply Recombinant technology</p>
 <p>Success from the first experiment Confirmed specificity</p>	 <p>Ethical standards compliant Animal-free production</p>

Anti-Occludin antibody [EPR20992] (ab216327)

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