abcam

Product datasheet

Anti-ZO1 tight junction protein antibody ab216880

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Overview

Product name Anti-ZO1 tight junction protein antibody

Description Rabbit polyclonal to ZO1 tight junction protein

Host species Rabbit

Tested applications
Suitable for: WB, ICC
Species reactivity
Reacts with: Human

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: Wild-type HeLa, U-2 OS, WM115, A549, HeLa, PC-3, and SK-OV-3 whole cell lysates. ICC:

Caco-2, MCF7 cells.

General notes

The Life Science industry has been in the grips of a reproducibility crisis for a number of years.

Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. 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, along with publications, customer reviews and Q&As

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 Preservative: 0.01% Sodium azide

Constituent: PBS

Purity Immunogen affinity purified

Clonality Polyclonal

Isotype IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab216880 in the following tested applications.

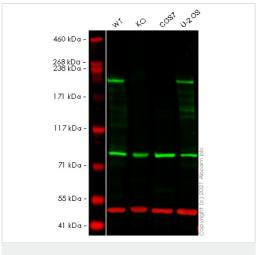
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The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		1/1000. Detects a band of approximately 195, 245 kDa (predicted molecular weight: 195 kDa). This antibody is more reactive to mouse in IHC than it is to mouse lysates in Western blot.
ICC		Use a concentration of 1 - 15 μg/ml.

Target		
Function	The N-terminal may be involved in transducing a signal required for tight junction assembly, while the C-terminal may have specific properties of tight junctions. The alpha domain might be involved in stabilizing junctions.	
Tissue specificity	The alpha-containing isoform is found in most epithelial cell junctions. The short isoform is found both in endothelial cells and the highly specialized epithelial junctions of renal glomeruli and Sertoli cells of the seminiferous tubules.	
Sequence similarities	Belongs to the MAGUK family. Contains 1 guanylate kinase-like domain. Contains 3 PDZ (DHR) domains. Contains 1 SH3 domain. Contains 1 ZU5 domain.	
Domain	The second PDZ domain mediates interaction with GJA12.	
Post-translational modifications	Phosphorylated. Dephosphorylated by PTPRJ.	
Cellular localization	Cell membrane. Cell junction > tight junction. Movement of ZO-1 from the cytoplasm to membrane is an early event occurring concurrently with cell-cell contact.	

Images



Western blot - Anti-ZO1 tight junction protein antibody (ab216880)

All lanes : Anti-ZO1 tight junction protein antibody (ab216880) at 1/1000 dilution

Lane 1: Wild-type HeLa cell lysate at 20 µg

Lane 2: TJP1 knockout HeLa cell lysate at 20 µg

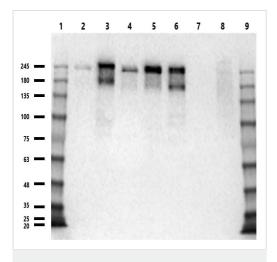
Lane 3: COS7 cell lysate at 10 μg **Lane 4**: U-2 OS cell lysate at 20 μg

Performed under reducing conditions.

Predicted band size: 195 kDa Observed band size: 200 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab216880 observed at 200 kDa. Red - loading control <u>ab7291</u> (Mouse anti-Alpha Tubulin [DM1A]) observed at 55 kDa.

ab216880 was shown to react with ZO1 tight junction protein in wild-type HeLa cells in Western blot with loss of signal observed in TJP1 knockout cell line ab264897 (TJP1 knockout cell lysate ab257744). Wild-type HeLa and TJP1 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with ab216880 and ab7291 (Mouse anti-Alpha Tubulin [DM1A]) overnight at 4 °C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit lgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse lgG H&L (IRDye® 680RD) preabsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.



Western blot - Anti-ZO1 tight junction protein antibody (ab216880)

All lanes : Anti-ZO1 tight junction protein antibody (ab216880) at 1 μ g/ml

Lanes 1 & 9: Protein Standard at 10 µl

Lane 2: WM115 (human melanoma cell line) whole cell lysate at 10 µq

Lane 3 : A549 (human lung carcinoma cell line) whole cell lysate at $10 \ \mu g$

Lane 4: HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate at 10 µg

Lane 5 : PC-3 (human prostate adenocarcinoma cell line) whole cell lysate at 10 μg

Lane 6 : SK-OV-3 (human ovarian cancer cell line) whole cell lysate at 10 μg

Lane 7: Mouse testis tissue lysate at 10 μg **Lane 8**: Rat testis tissue lysate at 10 μg

Secondary

All lanes: Goat anti Rabbit lgG H&L (HRP) at 1/70000 dilution

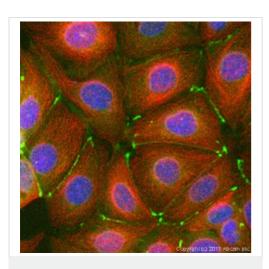
Performed under reducing conditions.

Predicted band size: 195 kDa

Observed band size: 195,245 kDa

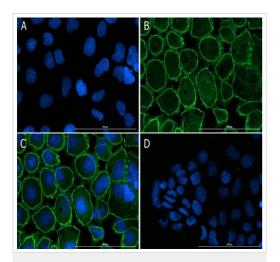
Exposure time: 5 seconds

Lysates reduced with beta-Mercaptoethanol and proteins separated by SDS-PAGE. Transferred to 0.2 μ m nitrocellulose membrane at 20V for 7 minutes. Blocked with 5% BLOTTO for 1 hour at room temperature. Incubated with primary overnight at 2-8°C, followed by secondary for 30 minutes at room temperature.



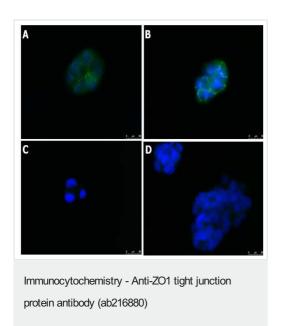
Immunocytochemistry - Anti-ZO1 tight junction protein antibody (ab216880)

ab216880 stained in MCF7 (Human breast adenocarcinoma cell line) cells. Cells were fixed with 100% methanol (5 minutes) at room temperature and incubated with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% tween for 1 hour at room temperature to permeabilize the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody ab216880 at 1 μ g/ml and ab7291 (Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control) at 1/1000 dilution overnight at +4°C. The secondary antibodies were ab150120 (pseudo-colored red) and ab150081 (colored green) used at 1 μ g/ml for 1 hour at room temperature. DAPI was used to stain the cell nuclei (colored blue) at a concentration of 1.43 μ M for 1 hour at room temperature



Immunocytochemistry - Anti-ZO1 tight junction protein antibody (ab216880)

Immunocytochemistry analysis of 4% paraformaldehyde fixed Caco-2 (human colorectal adenocarcinoma cell line) cells labeling ZO1 tight junction protein with ab216880 at 15 μ g/mL. Cells permeabilized with 0.3% Triton X-100 and incubated with primary antibody overnight at 2-8°C. Donkey Anti-Rabbit lgG DyLightTM 488 Conjugated (Preadsorbed) was used as the secondary antibody at 5 μ g/mL for 1 hour at room temperature. Staining: (A) DAPI. (B) ab216880 with secondary. (C) Merge A-B. (D) Secondary Only.



Immunofluorescent analysis of 0.5% PFA-fixed (A and C) and 0.5% methanol-fixed (B and D) Caco-2 (human colorectal adenocarcinoma cell line) cells labeling ZO1 tight junction protein with ab216880 at 10 μ g/ml (green).

The nuclear counter stain is DAPI (blue).

Secondary antibody only control: C and D.

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