

Datasheet for 600-401-GU7S**ZO-1 Antibody****Overview**

Description:	Anti-ZO-1 (RABBIT) Antibody - 600-401-GU7S
Item No.:	600-401-GU7S
Size:	25 µL
Applications:	ELISA, FC, IF, IHC, Multiplex, WB
Reactivity:	Human, Mouse
Host Species:	Rabbit

Product Details

Background:	ZO-1, also called TJP1, belongs to the MAGUK family. This gene encodes a protein located on a cytoplasmic membrane surface of intercellular tight junctions. The encoded protein may be involved in signal transduction at cell-cell junctions. 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. ZO-1 plays a role in the regulation of cell migration by targeting CDC42BPB to the leading edge of migrating cells. ZO1 may be associated the following disorders; celiac disease, congenital nephrotic syndrome finnish type, and macular degeneration. Anti-ZO-1 Antibody is useful for researchers interested in Apoptosis Research and Insulin Research.
Synonyms:	rabbit anti-ZO-1 antibody, ZO 1, ZO1, Tight junction protein ZO-1, Tight junction protein 1, Zona occludens protein 1, Zonula occludens protein 1, TJP1
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	TJP1
Reactivity:	Human, Mouse
Immunogen Type:	Conjugated Peptide

Immunogen:	Anti-ZO-1 antibody was prepared from whole rabbit serum produced by repeated immunizations with a synthetic peptide corresponding to an internal portion of human ZO-1 conjugated to Keyhole Limpet Hemocyanin (KLH).
Purity/Specificity:	This affinity purified antibody is directed against human ZO-1. This product was affinity purified from monospecific antiserum by immunoaffinity purification. Blast analysis of the sequence of the immunogen shows 100% identity with human.
Relevant Links:	• GeneID - 7082 • NCBI - NP_003248.3 • UniProtKB - Q07157

Application Details

Tested Applications:	ELISA, FC, IF, IHC, Multiplex, WB
Application Note:	Anti-ZO-1 Antibody has been tested in Western Blot, ELISA, Immunohistochemistry, Immunofluorescence, and Flow Cytometry. Expect a band at ~245 and/or 195.5 kDa in western blot using appropriate lysates. Positive control whole cell lysates used A549 and PC3 @ 1µg/mL for WB; CACO2 and PC3 with PFA and MeOH @ 10µg/mL for IF. Positive control cells for FC were PC3 and positive control tissues for IHC was mouse adipose tissue.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	10,000-1:50,000
FC:	User Optimized
IF:	10 µg/ml
IHC:	1:100 - 1:200
WB:	1:1000

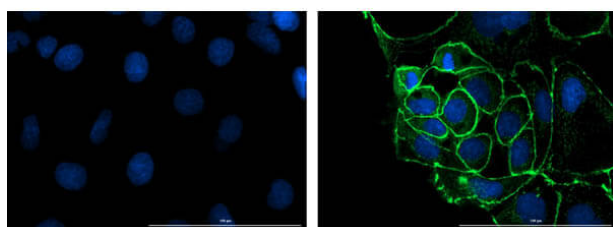
Formulation

Physical State:	Liquid (sterile filtered)
Concentration:	1.1 mg/ml by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	None

Shipping & Handling

Shipping Condition:	Dry Ice
Storage Condition:	Store vial at -20° C or below prior to opening. This vial contains a relatively low volume of reagent (25 µL). To minimize loss of volume dilute 1:10 by adding 225 µL of the buffer stated above directly to the vial. Recap, mix thoroughly and briefly centrifuge to collect the volume at the bottom of the vial. Use this intermediate dilution when calculating final dilutions as recommended below. Store the vial at -20°C or below after dilution. Avoid cycles of freezing and thawing.
Expiration:	Expiration date is one (1) year from date of receipt.

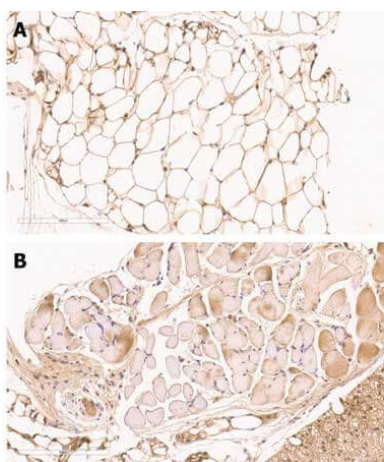
Images



Immunofluorescence Microscopy

Immunofluorescence microscopy of Anti-ZO-1 in Caco-2 cells using FITC-conjugated Fluorescent TrueBlot® anti-rabbit IgG (p/n 18-0216-32) for detection.

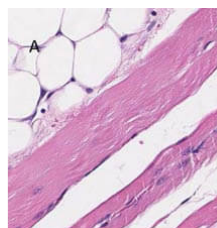
Caco-2 cells were fixed with 4% PFA, blocked (5% mouse serum/0.3% Triton X-100 in 1X PBS) for 1hr, then incubated with 15µg/mL of anti-ZO-1 primary antibody (Cat. No. 600-401-GU7) at 4°C overnight. Following 3 washes in 1X PBS for 5min each, 5µg/mL of FITC-conjugated Fluorescent TrueBlot® anti-rabbit IgG was added and allowed to incubate for 1hr at room temperature. Nuclei were counterstained with DAPI present in mounting medium. Predicted cell localization is cell membrane and cell junctions. Image taken at 40X magnification. (Right) Merged DAPI (blue)/ZO-1 (green), image shown (Left) secondary antibody only.



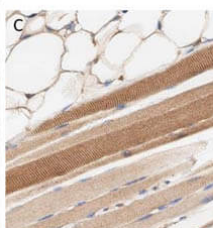
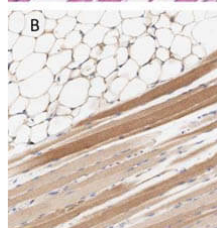
Immunohistochemistry

Immunohistochemistry of Rabbit anti-ZO-1 antibody. Tissue: mouse adipose tissue. Fixation: formalin fixed paraffin embedded. Epitope retrieval: heat induced (HIER). Primary antibody: ZO-1 antibody at 1:100 [A] and 1:200 [B] for 1 h at RT. Localization: ZO-1 will stain cell-cell junctions.

Visualized with WARP RED on MACH 4 universal AP polymer detection system.



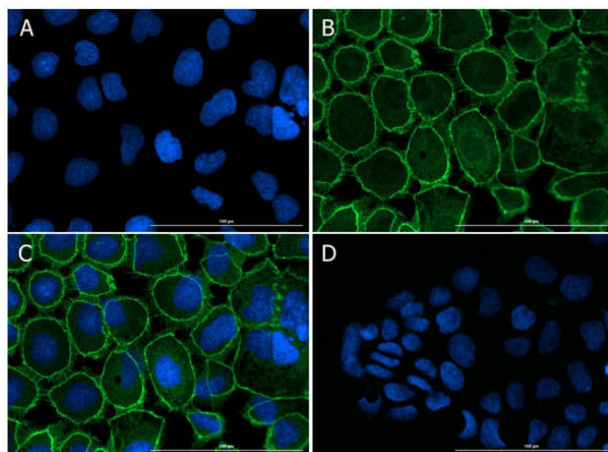
A. Mouse fat pad and muscle H&E
 B. Zo-1 at 20X, 1:100
 C. Zo-1 at 40X, 1:100



Immunohistochemistry

Immunohistochemistry of Rabbit Anti-ZO-1 antibody.

Tissue: mouse adipose tissue and muscle. Fixation: formalin fixed paraffin embedded. Antigen retrieval: heat induced (HIER) using Citrate Buffer for 20min. Primary antibody: ZO-1 antibody at 1:100 for 30min at RT. Secondary Antibody: Anti-Rabbit Poly-HRP-IgG Ready-to-Use for 8min at RT. Localization: ZO-1 will stain cell-cell junctions. Staining: DAB. Counter Stain: Hematoxylin.

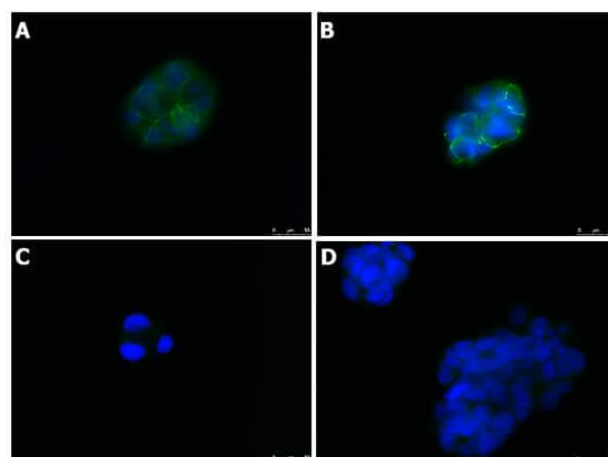


Immunofluorescence Microscopy

Immunofluorescence Microscopy of Rabbit anti-ZO-1 antibody. Tissue: CaCO2. Fixation: 4% PFA.

Permeabilization: 0.3%Triton X-100. Primary antibody: ZO-1 antibody at 15µg/mL overnight at 2-8°C. Secondary antibody: Donkey Anti-Rabbit IgG DyLight™ 488 Conjugated Preadsorbed (p/n 611-741-127) at 5µg/mL for 1 h at RT.

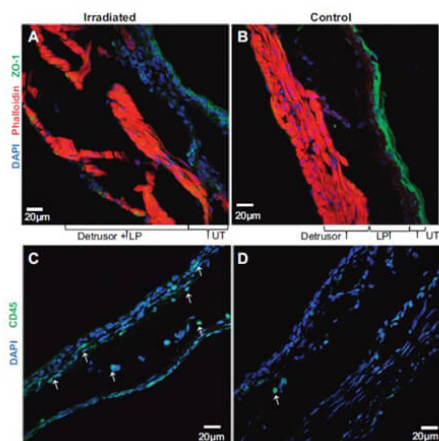
Localization: membrane. Staining: (A)DAPI. (B)ZO-1+DyLight488. (C)Merge A-B. (D) Secondary Only.



Immunofluorescence Microscopy

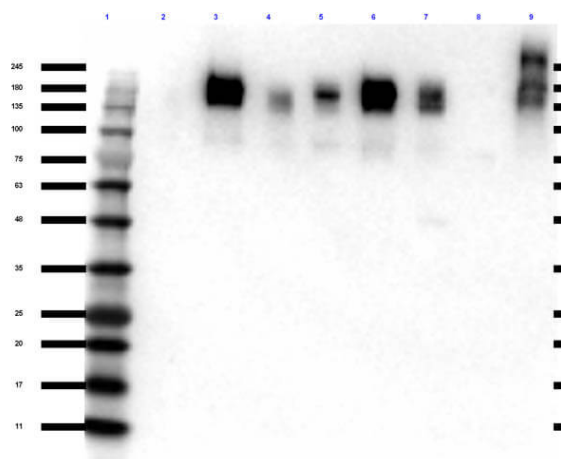
Immunofluorescence Microscopy of Rabbit anti-ZO-1 antibody. Tissue: Caco2. Fixation: 0.5% PFA [A,C]. 0.5% MeOH [B,D]. Antigen retrieval: not required. Primary antibody: ZO-1 antibody at 10 µg/mL for 1 h at RT.

Secondary antibody: Anti-RABBIT IgG DyLight™ 488 Conjugated Preadsorbed (p/n 611-741-127) at 5 ug/ml for 1 h at RT. Localization: (1) most epithelial cell junctions; (2) both in endothelial cells and the highly specialized epithelial junctions of renal and Sertoli cells. Staining: Target as green fluorescent signal with DAPI (blue) nuclear counterstain.



Immunofluorescence Microscopy

Immunofluorescence of ZO-1 and CD45. Representative confocal images of irradiated group (A) and controls (B) demonstrate a dramatic reduction of green fluorescence for ZO-1 in urothelium layer of irradiated group. Bladder sections were labeled with anti-ZO-1 antibodies and secondary donkey antibody Alexa 488 to localize the tight junction protein ZO-1 and with rhodamine-phalloidin to label the actin cytoskeleton (red) and DAPI to label the nuclei. Decreased expression of ZO-1 in urothelium layer of irradiated group was associated with an increase in CD45-positive, green fluorescent leukocytes (indicated by white arrows) in urothelium and lamina propria (C) compared with controls (D). Increased number of CD45-positive leukocytes indicates infiltration in irradiated bladder beyond the resident CD45 leukocytes spotted at one place in control section. Scale bar indicates magnification in different panels. Image Courtesy of Nishant Singh, Department of Urology, University of Pittsburgh, Pennsylvania.



Western Blot

Western Blot of Rabbit Anti-ZO-1 Antibody. Lane 1: Opal Pre-stained MW ladder (p/n MB-210-0500). Lane 2: WM-115 Whole Cell Lysate (p/n W09-001-GY8). Lane 3: A549 Whole Cell Lysate (p/n W09-001-372). Lane 4: HeLa Whole Cell Lysate (p/n W09-000-364). Lane 5: HeLa Whole Cell Lysate CCCP Stimulated (p/n W09-001-GZ0). Lane 6: PC-3 Whole Cell Lysate (p/n W09-001-GV6). Lane 7: SK-OV-3 Whole Cell Lysate (p/n W09-001-GX9). Lane 8: Mouse Testis Lysate (p/n W10-000-GZ2). Lane 9: Rat Testis Lysate (p/n W12-000-GZ3). Load: 10 µg per lane. Primary antibody: ZO-1 antibody at 1:1000 for overnight at 4°C. Secondary antibody: rabbit secondary HRP antibody (p/n 611-103-122) at 1:70,000 for 1 hr at RT. Block: 5% BLOTTO (p/n B501-0500) for 30 min at RT. Predicted/Observed size: ~187, 195 kDa for ZO-1.

References

- Singh N, Wang C, Cooper R. Virtual measurements of paracellular permeability and chronic inflammation via color coded pixel-wise T1 mapping. *Am J Physiol Renal Physiol.* (2020)

Disclaimer

This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information. All products of animal origin manufactured by Rockland Immunochemicals are derived from starting materials of North American origin. Collection was performed in United States Department of Agriculture (USDA) inspected facilities and all materials have been inspected and certified to be free of disease and suitable for exportation. All properties listed are typical characteristics and are not specifications. All suggestions and data are offered in good faith but without guarantee as conditions and methods of use of our products are beyond our control. All claims must be made within 30 days following the date of delivery. The prospective user must determine the suitability of our materials before adopting them on a commercial scale. Suggested uses of our products are not recommendations to use our products in violation of any patent or as a license under any patent of Rockland Immunochemicals, Inc. If you require a commercial license to use this material and do not have one, then return this material, unopened to: Rockland Inc., P.O. BOX 5199, Limerick, Pennsylvania, USA.