Anti-ZO1 Antibody [PSH07-09]

HA722797

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat, Monkey
Applications:	WB, IF-Cell, IHC-P
Molecular Wt:	Predicted band size: 195 kDa
Clone number:	PSH07-09

Description: Zonula occludens-1 ZO-1, also known as Tight junction protein-1 is a 220-kD peripheral membrane protein that is encoded by the TJP1 gene in humans. It belongs to the family of zonula occludens proteins (ZO-1, ZO-2, and ZO-3), which are tight junction-associated proteins and of which, ZO-1 is the first to be cloned. It was first isolated in 1986 by Stevenson and Goodenough using a monoclonal antibody raised in rodent liver to recognise a 225-kD polypeptide in whole liver homogenates and in tight junction-enriched membrane fractions. It has a role as a scaffold protein which cross-links and anchors Tight Junction (TJ) strand proteins, which are fibril-like structures within the lipid bilayer, to the actin cytoskeleton. 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. Two transcript variants encoding distinct isoforms have been identified for this gene.

Immunogen: Recombinant protein within human ZO1 aa 1,401-1,748.

Positive control: A431 cell lysate, 293T cell lysate, HepG2 cell lysate, HeLa cell lysate, U-2 OS cell lysate, NIH/3T3 cell lysate, C2C12 cell lysate, COS-1 cell lysate, Mouse testis tissue lysate, Rat testis tissue lysate, MCF7, human kidney tissue, mouse kidney tissue, rat kidney tissue.

Subcellular location: Cell membrane, Cell junction, tight junction, gap junction, Cell projection, podosome.

Database links: SwissProt: Q07157 Human | P39447 Mouse
Entrez Gene: 292994 Rat

Recommended Dilutions:

WB	1:1,000
IF-Cell	1:100
IHC-P	1:200

Storage Buffer: PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

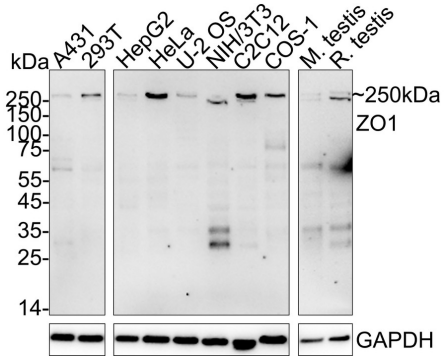

Fig1: Western blot analysis of ZO1 on different lysates with Rabbit anti-ZO1 antibody (HA722797) at 1/1,000 dilution.

Lane 1: A431 cell lysate (20 µg/Lane)
Lane 2: 293T cell lysate (20 µg/Lane)
Lane 3: HepG2 cell lysate (20 µg/Lane)
Lane 4: HeLa cell lysate (20 µg/Lane)
Lane 5: U-2 OS cell lysate (20 µg/Lane)
Lane 6: NIH/3T3 cell lysate (20 µg/Lane)
Lane 7: C2C12 cell lysate (20 µg/Lane)
Lane 8: COS-1 cell lysate (20 µg/Lane)
Lane 9: Mouse testis tissue lysate (40 µg/Lane)
Lane 10: Rat testis tissue lysate (40 µg/Lane)

Predicted band size: 195 kDa
Observed band size: 250 kDa

Exposure time: 3 minutes; ECL: K1802;
4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA722797) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

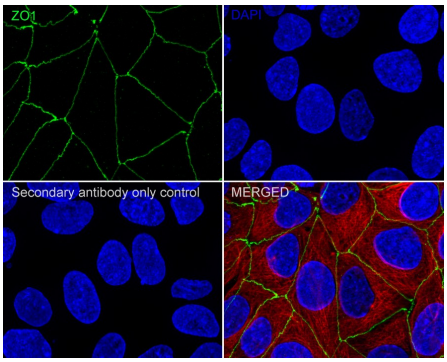

Fig2: Immunocytochemistry analysis of MCF7 cells labeling ZO1 with Rabbit anti-ZO1 antibody (HA722797) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-ZO1 antibody (HA722797) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

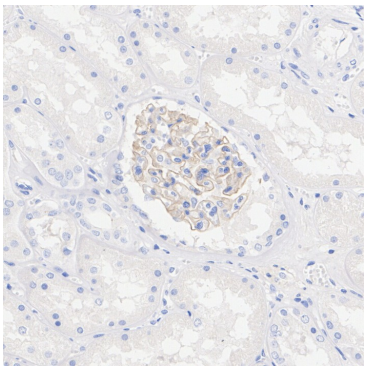

Fig3: Immunohistochemical analysis of paraffin-embedded human kidney tissue with Rabbit anti-ZO1 antibody (HA722797) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA722797) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

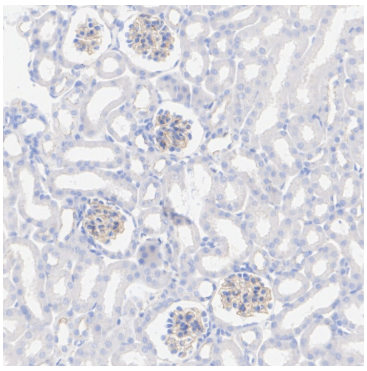

Fig4: Immunohistochemical analysis of paraffin-embedded mouse kidney tissue with Rabbit anti-ZO1 antibody (HA722797) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA722797) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

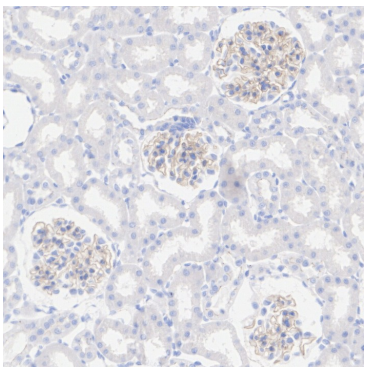

Fig5: Immunohistochemical analysis of paraffin-embedded rat kidney tissue with Rabbit anti-ZO1 antibody (HA722797) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA722797) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

Background References

1. Tsurudome Y et al. Decreased ZO1 expression causes loss of time-dependent tight junction function in the liver of ob/ob mice. Mol Biol Rep. 2022 Dec
2. Han F et al. GLTSCR1 coordinates alternative splicing and transcription elongation of ZO1 to regulate colorectal cancer progression. J Mol Cell Biol. 2022 Jun

Applications:WB=Western blot IHC-P=Immunohistochemistry (paraffin) IF-Cell=Immunofluorescence (Cell) IF-Tissue=Immunofluorescence (Tissue) FC=Flow cytometry IP=Immunoprecipitation