# abcam

# Product datasheet

# Anti-ZO1 tight junction protein antibody ab96587

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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: ICC/IF, WB

Unsuitable for: IHC-P

Species reactivity Reacts with: Mouse, Human

**Immunogen** Recombinant protein fragment corresponding to a region within amino acids 1 - 266 of Human

ZO1 tight junction protein.

Positive control WB: HEK-293T, HeLa, Neuro-2a, C8-D30, NIH/3T3 and C2C12 cell lysate, mouse testis tissue

lysate. ICC/IF: NTERA-2 cl.D1 [NT2/D1] cells and H661 xenograft tissue.

**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.

Avoid freeze / thaw cycle.

Storage buffer pH: 7.00

Preservative: 0.025% Proclin 300

Constituents: 79% PBS, 20% Glycerol (glycerin, glycerine)

**Purity** Immunogen affinity purified

**Clonality** Polyclonal

**Isotype** IgG

1

#### **Applications**

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab96587 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
ICC/IF		1/100 - 1/1000. Fix cells in ice-cold MeOH
WB	★★★★☆ (2)	1/500 - 1/3000. Predicted molecular weight: 187 kDa. 5% gel. Blocking condition: 5% non-fat milk in TBST, RT, 60min. Primary antibody incubation: 4°C overnight.
		Molecular weight shift may be caused by post-translation modification, there are multiple phosphorylation sites on ZO-1. It

**Application notes** 

Is unsuitable for IHC-P.

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**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.

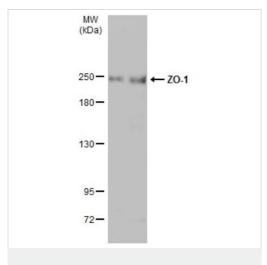
Post-translational modifications

Phosphorylated. Dephosphorylated by PTPRJ.

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 (ab96587)

**All lanes :** Anti-ZO1 tight junction protein antibody (ab96587) at 1/1000 dilution

Lane 1 : HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate

Lane 2 : HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate

Lysates/proteins at 30 µg per lane.

# **Secondary**

All lanes: HRP-conjugated anti-rabbit lgG antibody

Predicted band size: 187 kDa

5% gel.

Running condition: 80V, 15min; 140V, 40min.

Transfer condition:Semi-dry, 18 V, 60min (Nitrocellulose membrane)

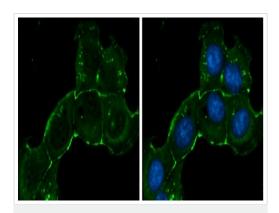
Blocking condition: 5% non-fat milk in TBST, RT, 60min.

Primary antibody incubation: 4°C overnight.

Secondary antibody incubation: Room temperature for 1 hour.

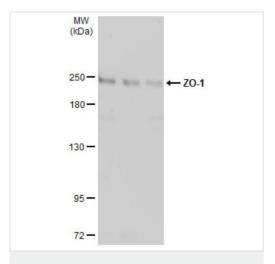
Washing condition: 5 ml TBST, 4 x 5min.

ECL detection.



Immunocytochemistry/ Immunofluorescence - Anti-ZO1 tight junction protein antibody (ab96587)

Immunofluorescence analysis of ice-cold methanol-fixed (5 min) NTERA-2 cl.D1 [NT2/D1] (human malignant pluripotent embryonic carcinoma cell line) cells labeling ZO1 tight junction protein at cell membrane (green) with ab96587 at 1/200 dilution. Blue: Hoechst 33342 staining.



Western blot - Anti-ZO1 tight junction protein antibody (ab96587)

**All lanes :** Anti-ZO1 tight junction protein antibody (ab96587) at 1/500 dilution

**Lane 1 :** Neuro-2a (mouse neuroblastoma cell line) whole cell lysate

**Lane 2**: C8-D30 (mouse astrocyte type III cerebellum cell line) whole cell lysate

**Lane 3**: NIH/3T3 (mouse embryo fibroblast cell line) whole cell lysate

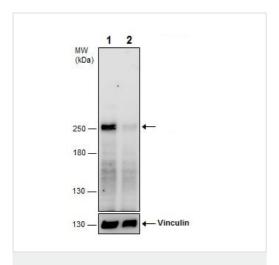
Lysates/proteins at 30 µg per lane.

# Secondary

All lanes: HRP-conjugated anti-rabbit IgG antibody

Predicted band size: 187 kDa

5% SDS-PAGE



Western blot - Anti-ZO1 tight junction protein antibody (ab96587)

**All lanes :** Anti-ZO1 tight junction protein antibody (ab96587) at 1/1000 dilution

**Lane 1 :** Non-transfected HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell extract

Lane 2 : ZO1 tight junction protein shRNA-transfected HeLa whole cell extract

Lysates/proteins at 30 µg per lane.

# **Secondary**

All lanes: HRP-conjugated anti-rabbit lgG

Predicted band size: 187 kDa

# 5% SDS-PAGE

MW (kDa)

250 — ← ZO-1

130 —

100 —

70 —

Western blot - Anti-ZO1 tight junction protein antibody (ab96587)

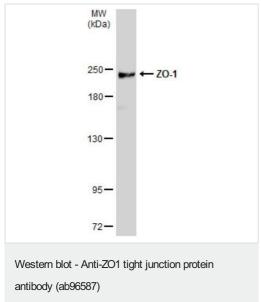
Anti-ZO1 tight junction protein antibody (ab96587) at 1/500 dilution + Mouse testis tissue lysate at 50  $\mu g$ 

# **Secondary**

HRP-conjugated anti-rabbit IgG antibody

Predicted band size: 187 kDa

5% SDS-PAGE



Anti-ZO1 tight junction protein antibody (ab96587) at 1/500 dilution + C2C12 (mouse myoblast cell line) whole cell lysate at 30 µg

# Secondary

HRP-conjugated anti-rabbit IgG antibody

Predicted band size: 187 kDa

5% SDS-PAGE

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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