

ZO-1 Ready-To-Use IHC Kit

Cat.No: IHC0641
Applications: **IHC-P**
Reactivity: Human, Mouse, Rat
Size: 50T
Assay type: Immunohistochemistry
Sample type: FFPE tissue
General Information:

Number	Component	Size	Concentration	Storage
1	PBS Buffer (powder)	2 L X 2	20x	RT
2	Antigen Retrieval Buffer	20 ml	100x	2-8°C
3	Endogenous Peroxidase Blocking Buffer	3 ml	RTU	2-8°C, protect from light
4	Blocking Buffer	3 ml	RTU	2-8°C
5	Primary Antibody (ZO-1 Recombinant Rabbit mAb)	6 ml	RTU	2-8°C
6	Secondary Antibody (Goat Anti-Rabbit IgG H&L, HRP conjugated)	6 ml	RTU	2-8°C
7	Chromogen Component A	0.3 ml	RTU	-20°C, protect from light
8	Chromogen Component B	0.3 ml	RTU	-20°C
9	Counter Staining Reagent	5 ml	RTU	RT
10	Mounting Media	5 ml	RTU	RT
11	Control slide (Human kidney, rat kidney, mouse kidney)	3 slides	RTU	RT
12	Datasheet	1 copy		

Storage and Stability: Please store components at the temperatures indicated on the individual tube labels. The kit is stable for 6 months from the date of receipt.

Immunohistochemistry Protocol:

1. Deparaffinization And Rehydration

Immerse slides in fresh xylene for 15 minutes and then repeat two more times using separate containers. Immerse slides sequentially in 100%, 95%, 90%, 80%, and 70% ethanol solutions for 5 minutes each. Rinse slides 3 times with distilled water for 5 minutes each.

2. Antigen Retrieval (Pressure Cooker)

Prepare a 1x antigen retrieval solution by diluting the 100x Antigen Retrieval Buffer using distilled water. Add the appropriate amount of 1x antigen retrieval solution into the pressure cooker and place a heat-resistant staining container filled with the same solution inside the cooker. Heat the solution to boiling with the lid of the pressure cooker rested on

top without being secured. Once it's boiling, transfer the slides from the distilled water to the staining container inside the pressure cooker. Follow the manufacturer's instructions to secure the lid of the pressure cooker. As soon as the cooker reaches full pressure, time three minutes. After three minutes, move the pressure cooker to an empty sink and cool it down by running cold water over the cooker. Once depressurized, open the lid and transfer the staining container with the slides to room temperature. After 20 minutes, rinse 3 times with PBS Buffer (dissolve the powder in 2L distilled water) for 5 minutes each.

3. **Block Endogenous Peroxidase**

Drain the liquid off the slides and then use a hydrophobic IHC pen to draw circles on the slides around tissue sections. Add 2-4 drops of **Endogenous Peroxidase Blocking Buffer** directly on slides, covering the whole tissue and block slides for 15 minutes at RT. Rinse 3 times with **PBS Buffer** for 5 minutes each.

4. **Serum Blocking**

Block with 2-4 drops of **Blocking Buffer** for 20 minutes at RT.

5. **Primary Antibody Incubation**

Drain blocking buffer from slides. Incubate slides with 2-4 drops of **ZO-1 Recombinant Rabbit mAb** overnight at 4°C or 1-2 hours at RT. Rinse 3 times with **PBS Buffer** for 5 minutes each.

6. **Secondary Antibody Incubation**

Incubate slides with 2-4 drops of **Goat Anti-Rabbit IgG H&L, HRP conjugated** for 1-2 hours at RT. Rinse slides 3 times with **PBS Buffer** for 5 minutes each.

7. **Signal Development**

Remove residual liquid around the tissue section. Add 50ul fresh **DAB Buffer (Chromogen Component A : Chromogen Component B : PBS Buffer=1:1:18)** to cover the tissue. Monitor the reaction under the microscope until a brown color is visible (approximate 3-5 minutes at RT). Stop reaction immediately by rinsing with distilled water. Rinse slides 3 times with distilled water for 5 minutes each.

8. **Counterstain**

Counterstain with an appropriate amount of **Counter Staining Reagent** for 3-5 minutes at RT. Rinse slides with distilled water for 5 minutes. Use 2-4 drops of **Differentiation reagent** to cover the tissue for 30 seconds. Rinse slides twice with distilled water for 5 minutes each.

9. **Dehydration Sheet**

Immerse slides sequentially in 70%, 80%, 90%, 95%, and 100% ethanol for 5 minutes each at RT. Immerse slides in 2 changes of fresh xylene, 15 minutes each. Drop some **Mounting Media** on the tissue. Mount coverslips.

Notes:

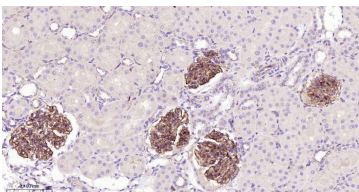
1. The positive control slide provided in the kit allows you to be sure that the experimental set-up is working properly.
2. Do not allow slides to dry at any time during this procedure.
3. Please don't replace the matching reagents in this product with other manufacturers' products.
4. As DAB is a carcinogen, please take necessary precautions.
5. PBS (reagent 1) can be stored for one week at 4°C after preparation; The antigen retrieval buffer (1× reagent 2) and the chromogenic agent (the mixture of reagents 7 and 8) should be prepared right before each assay.

Please cite this product as "IHC0641, Bioss Antibodies". Citation example: "Tissue sections using TJP1 IHC Kit (IHC0641, Bioss Antibodies) were stained for TJP1 according to the manufacturer's instructions."

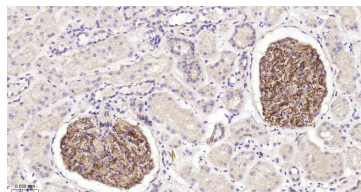
Introduction:

ZO-1 forms complexes with either ZO-2 or ZO-3. In addition, these proteins can also associate with claudin, occludin and F-actin, at tight junction stands, where they provide a linkage between the actin cytoskeleton and the tight junction. ZO-1 expression is significantly reduced in many breast cancer lines. ZO-2 and ZO-3 are ubiquitously expressed within epithelial tight junctions, and unlike ZO-1, which is also expressed at cell junctions of cardiac myocytes, ZO-2 is not expressed in nonepithelial tissue. ZO-1 is a protein located on a cytoplasmic membrane surface of intercellular tight junctions. ZO-1 may be involved in signal transduction at cell-cell junctions. Two transcript variants encoding distinct isoforms have been identified for the ZO-1 gene. Diseases associated with ZO-1 dysfunction include Celiac Disease and Congenital Nephrotic Syndrome.

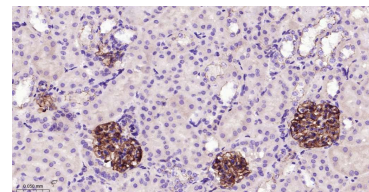
Validation Data



Immunohistochemical analysis of paraffin embedded Rat kidney tissue slide using IHC0641 (ZO-1 Kit).



Immunohistochemical analysis of paraffin embedded Human kidney tissue slide using IHC0641 (ZO-1 Kit).



Immunohistochemical analysis of paraffin embedded Mouse kidney tissue slide using IHC0641 (ZO-1 Kit).