

Detection of ZO-1 in Formalin-Fixed, Paraffin-Embedded Rat Tissue

Reagent and Antibody Information

[1X Wash Buffer](#)

[3% Hydrogen Peroxide](#)

[1% BSA Diluent](#)

[Protease](#)

[DAB Chromagen](#)

[Hematoxylin](#)

Blocking Serum: Normal Donkey Serum

Jackson ImmunoResearch Laboratories, Inc.

West Grove, PA 19390

www.jacksonimmuno.com

1-800-367-5296

Catalog # 017-000-001

Avidin / Biotin Blocking Kit

Vector Laboratories, Inc.

Burlingame, CA 94010

www.vectorlabs.com

1-800-227-6666

Catalog # SP-2001

Primary Antibody: Rabbit Anti-ZO-1 Antibody

Invitrogen

Carlsband, CA 92008

www.invitrogen.com

1-800-955-6288

Catalog # 61-7300

Negative Control Serum: Normal Rabbit Serum

Jackson ImmunoResearch Laboratories, Inc.

West Grove, PA 19390

www.jacksonimmuno.com

1-800-367-5296

Catalog # 011-000-001

Secondary Antibody: Biotin-Conjugated Donkey Anti-Rabbit IgG

Jackson ImmunoResearch Laboratories, Inc.

West Grove, PA 19390

www.jacksonimmuno.com

1-800-367-5296

Catalog # 711-065-152

Label Complex: Vectastain Elite ABC Kit (Standard)

Vector Laboratories, Inc.

Burlingame, CA 94010

www.vectorlabs.com

1-800-227-6666

Catalog # PK-6100

Staining Procedure

Positive Control Tissue: Kidney (capillary loops in glomeruli)

Stain Localization: Cytoplasmic

1. Deparaffinize and hydrate slides through the following solutions:

Solution	Repetitions	Time
Xylene	2 times	5 minutes
100% Ethanol	2 times	3 minutes
95% Ethanol	2 times	3 minutes
1X Wash Buffer	2 times	5 minutes

2. Quench endogenous peroxidase by placing the slides in 3% hydrogen peroxide for 15 minutes.

3. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.

4. Proteolytic-Induced Epitope Retrieval Using Protease

Preheat slides to 37°C in 1X Wash Buffer.

Incubate the slides in a 0.01% protease solution (made in distilled water) for 5 minutes at 37°C.

Rinse the slide in distilled water for 1 minute to stop the enzymatic reaction.

5. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each time.

6. Block with 10% Normal Donkey Serum for 20 minutes at room temperature.

Lot # _____ Date Reconstituted _____

DO NOT RINSE SLIDES. CONTINUE TO AVIDIN-BIOTIN BLOCK.

7. Avidin / Biotin Blocking Kit

Lot # _____ Exp Date _____ New Kit: yes / no

Apply avidin block for 15 minutes at room temperature.

Quick rinse in 1X Wash Buffer.

Apply biotin block for 15 minutes at room temperature.

DO NOT RINSE SECTIONS WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.
ONLY WIPE EXCESS BLOCK.

8. Apply primary antibody at a 1:25 dilution, and incubate for 1 hour at room temperature.

Lot # _____ Exp Date _____

For negative control slides, dilute the protein concentration of the normal rabbit serum to match that of the primary antibody. Make a 1:25 dilution from this normalized serum, and apply to the slides.

Incubate for 1 hour at room temperature.

Lot # _____ Date Reconstituted _____

9. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.
10. Apply the donkey anti-rabbit secondary antibody at a 1:1000 dilution, and incubate for 30 minutes at room temperature.
Lot # _____ Date Reconstituted _____
11. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.
12. Apply the label complex from the Standard Elite Kit, and incubate for 30 minutes at room temperature. (Prepare at least 30 minutes prior to use.)
Exp. Date _____ New Kit: yes / no
13. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each time.
14. Apply the DAB chromagen, and incubate in the dark for 6 minutes at room temperature.
(Add 1 drop of DAB per ml of substrate)
Lot # _____ Exp. Date _____ New Kit: yes / no
15. Rinse the slides in tap water 3 minutes.
16. Counterstain with Harris Hematoxylin for 20 seconds.
17. Rinse the slides in tap water until water is clear.
18. Gently agitate slides in 1X Wash Buffer until they turn blue.
19. Dehydrate through the following solutions:

Solutions	Repetitions	Time
95% Ethanol	1 time	3 minutes
100% Ethanol	3 times	3 minutes
Xylene	2 times	5 minutes

20. Coverslip