

Detection of Dss1 in Formalin-Fixed, Paraffin-Embedded, Mouse Tissue

Reagents:

[1X Automation Buffer](#)
[3% Hydrogen Peroxide](#)
[Antibody Diluent](#)
[Citrate Buffer](#)
[DAB Chromagen](#)
[Hematoxylin](#)

Antibody Dilutions:

Kit: Vector Elite Rabbit IgG ABC kit
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog # PK 6101.

Avidin Biotin Blocking Kit
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog: #SP-2001

Primary Antibody
Polyclonal Antibody: Rabbit anti-Dss1
Antibody provided by: Carol Trempus

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

Staining Procedure

Positive Control Tissue: skin papilloma

Stain Localization: Cytoplamic

Deparaffinize and hydrate slides through the following solutions.

Xylene	2 times	5 minutes
100% EtOH	2 times	3 minutes
95% EtOH	2 times	3 minutes
1X Automation Buffer	2 times	5 minutes

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

2. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

3. Unmasking Techniques using the decloaker.

Add 500 ml D/W to the pan of the decloaker.

Place full rack of slides in 200 ml of 1X citrate buffer and place in the decloaker.

Decloak for 5 minutes. Pressure_____

Depressurize for 10 minutes.

Remove pan top and cool for 10 min. Temp_____

Rinse in D/W, 2x for 3 min each

4. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

5. Block in Vector Elite Rabbit block from the kit for 20 min.

Exp. Date_____ New Kit: yes / no

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

6. Apply Avidin/Biotin block

Lot#_____ Exp Date_____ New Kit: yes / no

Apply avidin block - 15 min at RT.

Quick rinse in 1X AB.

Apply biotin block - 15 min at RT.

Wipe excess block

DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.

7. Apply primary antibody (Dss1) at a 1:50 dilution and incubate for 1hr.

Lot#_____ Aliquoted yes / no Date Aliquoted_____

For negative control slides, normalize the protein concentration of the normal rabbit serum to the protein concentration of the primary antibody (Dss1) and use this to make the 1:50 dilution and incubate for one hour.

Lot# _____ Reconstituted Date _____

8. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.
 9. Apply secondary antibody from Vector Elite kit and incubate for 30 minutes.
 10. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.
 11. Apply Label antibody from Vector Elite kit and incubate for 30 minutes.
 12. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.
 13. Apply liquid Dako DAB Chromagen for 6 minutes in the dark.
(Add 1 drop of DAB per ml of substrate)
- Lot# _____ Exp. Date _____ New Kit yes / no
14. Rinse in tap water 3 minutes.
 15. Counterstain with Modified Harris Hematoxylin for 30 seconds.
 16. Rinse in tap water until water is clear.
 17. Place slides in 1X Automation buffer for 1 minute with gentle agitation to blue slides.
 18. Dehydrate through the following solutions.

95% alcohol	1 times	3 mins
100% alcohol	3 times	3 mins
Xylene	2 times	5 mins

19. Coverslip
updated 8/25/04