Detection of Thrombospondin in Formalin Fixed, Paraffin-Embedded Human Tissue

Reagents:

1X Automation Buffer
3% Hydrogen Peroxide
Antibody Diluent
EDTA
DAB Chromagen
Hematoxylin

Antibody Information

Kit: Vector Mouse Elite Kit
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog #PK-6102
*This kit contains all reagents necessary to make blocking, secondary and label antibodies.

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

Primary antibody: Mouse anti-thrombospondin
Lab Vision
Fremont, CA 94539 USA
www.labvision.com
1-800-828-1628
Catalog# MS-421-B

Negative Control: Normal Mouse Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog #015-000-001

Staining Procedure

-Positive Control Tissue: platelets (tonsil)
-Stain localization: Nuclear

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 EDTA (1:5) 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. Apply blocking solution from Vector Mouse Kit for 20 minutes at room temperature.
Exp. Date_____ New Kit: yes / no

DO NOT RINSE SECTIONS WITH BUFFER.

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 (TSP) at 1:1000 dilution and incubate for one hour at room temperature.
Lot#_____ Exp Date_____

For negative control slides, normalize the protein concentration of the normal mouse serum to the protein concentration of the primary antibody (TSP) and use this to make the 1:1000 dilution. Apply to slides and incubate for one hour at room temperature.
Lot #_____ Reconstituted Date_____

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

9. Apply the secondary antibody and incubate for 30 minutes.

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

11. Apply the label antibody and incubate for 30 minutes.
(Prepare at least 30 mins prior to use)

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% Ethanol	1 change	3 minutes
100% EtOH	3 changes	3 minutes
Xylene	2 changes	5 minutes

19. Coverslip
updated 01/20/05