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

Reagent and Antibody Information

1X Wash Buffer
3% Hydrogen Peroxide
1% BSA Diluent
1X EDTA
DAB Chromogen
Hematoxylin

Staining Kit: Vectastain Elite ABC Kit (Mouse IgG)
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog # PK-6102

Note: This kit contains all reagents necessary to make the blocking reagent, secondary antibody and label complex.

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

Primary Antibody: Mouse Monoclonal Anti-Thrombospondin Antibody
Lab Vision / Thermo Fisher Scientific
Fremont, CA 94539
www.labvision.com
1-800-828-1628
Catalog # MS-421

Negative Control Serum: Purified Mouse IgG1 Isotype Control Serum
BD Biosciences
San Jose, CA 95131
www.bdbiosciences.com
1-855-236-2772
Catalog # 557273
**Staining Procedure**

Positive Control Tissue: Tonsil platelets  
Stain Localization: Nuclear

1. Deparaffinize and hydrate slides through the following solutions:

<table>
<thead>
<tr>
<th>Solution</th>
<th>Repetitions</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xylene</td>
<td>2 times</td>
<td>5 minutes</td>
</tr>
<tr>
<td>100% Ethanol</td>
<td>2 times</td>
<td>3 minutes</td>
</tr>
<tr>
<td>95% Ethanol</td>
<td>2 times</td>
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<td>1X Wash Buffer</td>
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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. **Heat-Induced Epitope Retrieval Using The Decloaker**  
   Add 500 ml of distilled water to the pan inside the decloaker.  
   Place a full rack of slides into a Tissue Tek® container with 200 ml of 1X EDTA  
   (Insert blank slides into any empty slots in the rack to ensure even heating of slides)  
   Place the container stably inside the pan and decloak for 5 minutes.  
   Maximum Pressure ________  
   Depressurize for 10 minutes.  
   Remove pan top and cool for 10 minutes. **Temperature Before Cooling Slides________**  
   Rinse the slides in 2 changes of distilled water for 3 minutes each time.

5. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each time.

   Vectastain Mouse Elite Staining Kit  
   Exp. Date_______________ New Kit: yes / no

6. Apply the blocking solution from the Mouse Elite Kit. Incubate for 20 minutes at room temperature.  
   **DO NOT RINSE THE 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 SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.**  
   **ONLY WIPE EXCESS BLOCK.**

8. Apply primary antibody at a 1:1000 dilution. Incubate for 1 hour at room temperature.  
   Lot #_______________ Exp. Date _______________
For negative control slides, dilute mouse IgG1 control serum so that its IgG1 protein concentration matches that of the primary antibody (if necessary). Then make a 1:1000 dilution. If the concentrations can’t be matched using this method, the dilution for the negative reagent may need to be adjusted. Apply the negative and incubate for 1 hour at room temperature.
Lot #_______________ Exp. Date ________________

9. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.

10. Apply the secondary antibody from Mouse Elite Kit. Incubate for 30 minutes at room temperature.

11. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.

12. Apply the label complex from the Mouse Elite Kit. Incubate for 30 minutes at room temperature.

13. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.

14. Apply the DAB chromogen. 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 hematoxylin for 20 seconds.

17. Rinse the slides in tap water until water is clear.

18. Gently agitate slides in 1X wash buffer until the tissues turn blue.

19. Dehydrate through the following solutions:

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20. Coverslip

   Updated 01/20/05