Detection of S100 in Formalin-Fixed, Paraffin-Embedded in Rat Tissue

Reagent and Antibody Information

1X Wash Buffer
3% Hydrogen Peroxide
1% BSA Diluent
Normal Rabbit IgG – Affinity Purified
DAB Chromogen
Hematoxylin

Blocking Serum: Normal Goat Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog # 005-000-121

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

Primary Antibody: Rabbit Polyclonal Antibody To S100 Protein (Ab-2)
Lab Vision / Thermo Fisher Scientific
Fremont, CA 94539
www.labvision.com
1-800-828-1628
Catalog # RB-044

Secondary Antibody: Biotinylated Goat Anti-Rabbit IgG (H+L)
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog # BA-1000

Label Complex: R.T.U. Vectastain Elite ABC Reagent
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog # PK-7100
Staining Procedure

Positive Control Tissue: Skin
Stain Localization: Cytoplasmic

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>
<td>3 minutes</td>
</tr>
<tr>
<td>1X Wash Buffer</td>
<td>2 times</td>
<td>5 minutes</td>
</tr>
</tbody>
</table>

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. Block with 5% normal goat serum for 20 minutes at room temperature.
   Lot #_________________ Date Reconstituted_________________

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

5. 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.

6. Apply the primary antibody at a 1:500 dilution. Incubate for 30 minutes at room temperature.
   Lot #_________________ Exp Date __________________

   For negative control slides, dilute normal rabbit IgG so that it’s IgG protein concentration matches that of the primary antibody (if necessary). Then make a 1:500 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 30 minutes at room temperature.
   Lot #_________________ Exp. Date __________________

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

8. Apply the goat anti-rabbit secondary antibody at a 1:500 dilution. Incubate for 30 minutes at room temperature.
   Lot #_________________ Date Reconstituted_________________

9. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.
10. Apply the Vectastain R.T.U. Elite Label and incubate for 30 minutes at room temperature.
   Exp. Date______________ New Kit: yes / no

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

12. 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

13. Rinse the slides in tap water 3 minutes.


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

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

17. Dehydrate through the following solutions:

<table>
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<tr>
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<th>Repetitions</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>95% Ethanol</td>
<td>1 time</td>
<td>3 minutes</td>
</tr>
<tr>
<td>100% Ethanol</td>
<td>3 times</td>
<td>3 minutes</td>
</tr>
<tr>
<td>Xylene</td>
<td>2 times</td>
<td>5 minutes</td>
</tr>
</tbody>
</table>

18. Coverslip

*Updated 11/04/10*

This protocol has been made available for use by others. We cannot guarantee optimal results, so staining conditions must be determined by the end user. Protocol modifications may be required due to lot number changes and/or reagent substitutions.