Detection of Gastric Mucin in Formalin-Fixed, Paraffin-Embedded Mouse Tissue

Reagent and Antibody Information

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

Blocking Serum: Normal Horse Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog # 008-000-001

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-Mucin Gastric Antibody
Sigma-Aldrich
St. Louis, MO
www.sigmaaldrich.com
1-800-325-3010
Catalog # M5293

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

Secondary Antibody: Biotinylated Horse Anti-Mouse IgG (H+L)
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog # BA-2001
Staining Procedure

Positive Control Tissue: Gastrointestinal tract - stomach
Stain Localization: Cytoplasm of goblet cells

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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<tr>
<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 0.1 M citrate buffer
   (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.

6. Block with 10% normal horse 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 1:10,000 dilution. Incubate for 15 minutes at room temperature.
   Lot #______________ Exp. Date ____________________

For negative control slides, dilute mouse IgG1 control serum so that it’s IgG1 protein concentration matches that of the primary antibody (if necessary). Then make a 1:10,000 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 15 minutes at room temperature.
   Lot #______________ Exp. Date ____________________

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

10. Apply the horse anti-mouse secondary antibody at a 1:1000 dilution. 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 Vectastain R.T.U. Elite Label and incubate for 30 minutes at room temperature.
    Exp. Date______________ New Kit: yes / no

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 01/12/10

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.