Detection of SV40 Large T Antigen 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: Purified Mouse Anti-SV40 Large T Antigen Monoclonal Antibody
BD Biosciences
San Jose, CA 95131
www.bdbiosciences.com
1-855-236-2772
Catalog # 554149

Negative Control Serum: Purified Mouse IgG2a Isotype Control Serum
BD Biosciences
San Jose, CA 95131
www.bdbiosciences.com
1-855-236-2772
Catalog # 550339

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: Multiple tissues
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>
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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 slides in 2 changes of 1X wash buffer for 5 minutes each.

4. Heat-Induced Epitope Retrieval Using The Steamer
   Add distilled water to the bottom portion of the steamer.
   Preheat 200ml of 1X citrate buffer in a Tissue Tek® container in the steamer between 95ºC and 100ºC.
   Immerse a full rack of slides into the citrate buffer and place the lid back on the steamer.
   (Insert blank slides into any empty slots in the rack to ensure even heating of slides)
   Steam the slides for 20 minutes.
   Remove container from steamer and cool for 20 minutes.
   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 5% 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 1:100 dilution. Incubate for 1 hour at room temperature.
   Lot #____________________ Exp. Date____________________

For negative control slides, dilute mouse IgG2a control serum so that it’s IgG2a protein concentration matches that of the primary antibody (if necessary). Then make a 1:100 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 time.

10. Apply the horse anti-mouse secondary antibody at a 1:500 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 and 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

Update 08/08/03

National Institute of Environmental Health Sciences / NIH ● Immunohistochemistry Support Group

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.