Detection of Terminal Deoxynucleotidyl Transferase (TdT) in Formalin-Fixed, Paraffin-Embedded Mouse Tissue

Reagent and Antibody Information

1X Wash Buffer
3% Hydrogen Peroxide
1% BSA Diluent
1X EDTA
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 Anti-Terminal Deoxynucleotidyl Transferase (TdT)
Dakocytomation Corporation
Carpinteria, CA 93013
www.dako.com
1-800-235-5763
Code No. A3524

Negative Control Serum: Rabbit Immunoglobulin Fraction (Solid-Phase Adsorbed)
Dakocytomation Corporation
Carpinteria, CA 93013
www.dako.com
1-800-235-5763
Catalog # X0936

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
Staining Procedure

Positive Control Tissue: Thymus (T-cells)
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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<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 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.

6. Block with 10% normal goat 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 1:20 dilution. Incubate for 1 hour 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 1:20 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 goat anti-rabbit 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 time.

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 time.

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 10/05/04