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

Reagent and Antibody Information

1X Wash Buffer
3% Hydrogen Peroxide
1% BSA Diluent
DAB Chromagen
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: Goat Anti-Rat IgM Antibody HRP-Conjugated
Bethyl Laboratories, Inc
Montgomery, TX 77356
www.bethyl.com
1-800-338-9579
Catalog # A110-100P

Staining Procedure

Positive Control Tissue: Lymph Node
Stain Localization: Cytoplasmic (Secreted)

1. Deparaffinize and hydrate slides through the following solutions:

Solution	Repetitions	Time
Xylene	2 times	5 minutes
100% Ethanol	2 times	3 minutes
95% Ethanol	2 times	3 minutes
1X Wash Buffer	2 times	5 minutes

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. Block with 10% 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 primary antibody at a 1:200 dilution, and incubate for overnight at 4°C.
Lot #_____ Exp. Date _____

**********Next Day**********

7. Bring the slides up to room temperature in 1X Wash Buffer for at least 15 minutes.

8. Apply the DAB chromagen, 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

9. Rinse the slides in tap water 3 minutes.

10. Counterstain with Harris Hematoxylin for 20 seconds.

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

12. Gently agitate slides in 1X Wash Buffer until they turn blue.

13. Dehydrate through the following solutions:

Solutions	Repetitions	Time
95% Ethanol	1 time	3 minutes
100% Ethanol	3 times	3 minutes
Xylene	2 times	5 minutes

14. Coverslip

Updated 02/19/09