Detection of IgG in Formalin-Fixed, Paraffin-Embedded Mouse Tissue

Reagent and Antibody Information

1X Wash Buffer
3% Hydrogen Peroxide
1% BSA Diluent
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: Biotinylated Goat Anti-Mouse IgG (H+L)
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog # BA-9200

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: Spleen/thymus or gastrointestinal tract
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 the 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 1:1500 dilution. Incubate for 1 hour at room temperature.
   Lot #_____ Exp. Date _____

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

8. Apply the Vectastain R.T.U. Elite Label and incubate for 30 minutes at room temperature.
   Exp. Date_____ New Kit: yes / no

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

10. 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

11. Rinse the slides in tap water 3 minutes.

12. Counterstain with hematoxylin for 20 seconds.

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

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

15. 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

16. Coverslip

Updated 02/11/10