

Detection of IgA 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 IgA Antibody HRP-Conjugated

Bethyl Laboratories, Inc

Montgomery, TX 77356

www.bethyl.com

1-800-338-9579

Catalog # A110-102P

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 1:500 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