

# Detection of IgA 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](http://www.jacksonimmuno.com)

1-800-367-5296

Catalog # 005-000-121

Avidin / Biotin Blocking Kit

Vector Laboratories, Inc.

Burlingame, CA 94010

[www.vectorlabs.com](http://www.vectorlabs.com)

1-800-227-6666

Catalog # SP-2001

Primary Antibody: Biotinylated Goat Anti-Mouse IgA ( $\alpha$ ) Polyclonal Antibody

KPL

Gaithersburg, MD 20878

[www.kpl.com](http://www.kpl.com)

1-800-638-3167

Catalog # 16-18-01

Label Complex: R.T.U. Vectastain Elite ABC Reagent

Vector Laboratories, Inc.

Burlingame, CA 94010

[www.vectorlabs.com](http://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 1:500 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 02/16/10*