

# Detection of Myeloperoxidase in Formalin-Fixed and Bouins-Fixed, Paraffin-Embedded Mouse and Rat Tissue

## Reagent and Antibody Information

[1X Wash Buffer](#)

[3% Hydrogen Peroxide](#)

[1% BSA Diluent](#)

[1X Citrate Buffer](#)

[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: Rabbit Polyclonal Anti-Human Myeloperoxidase Antibody

Dakocytomation Corporation

Carpinteria, CA 93013

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

1-800-235-5763

Code No. A0398

### Negative Control Serum: Rabbit Immunoglobulin Fraction (Solid-Phase Adsorbed)

Dakocytomation Corporation

Carpinteria, CA 93013

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

1-800-227-6666

Catalog # BA-1000

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 or embryonic liver - neutrophils  
Stain Localization: Cytoplasmic

1. Deparaffinize and hydrate slides through the following solutions:

<b>Solution</b>	<b>Repetitions</b>	<b>Time</b>
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. Heat-Induced Epitope Retrieval Using The Steamer

Add distilled water to the bottom portion of the steamer.

Preheat 200ml of 1X citrate buffer in a Tissue Tek® container in the steamer between 95°C and 100°C.

Immerse a full rack of slides into the citrate buffer and place the lid back on the steamer.

(Insert blank slides into any empty slots in the rack to ensure even heating of slides)

Steam the slides for 20 minutes.

Remove container from steamer and cool for 20 minutes.

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 1:1200 dilution. Incubate for 30 minutes 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 1:1200 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 30 minutes at room temperature.

Lot # \_\_\_\_\_ Exp. Date \_\_\_\_\_

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

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

20. Coverslip

*Updated 08/14/12*