

# Detection of Hemoglobin 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 Donkey Serum

Jackson ImmunoResearch Laboratories, Inc.

West Grove, PA 19390

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

1-800-367-5296

Catalog # 017-000-011

### 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 Hemoglobin $\beta/\gamma/\delta$ Antibody (H-76)

Santa Cruz Biotechnology

Santa Cruz, CA 95060

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

1-800-457-3801

Catalog # sc-21006

### Negative Serum Control: Normal Rabbit Serum

Jackson ImmunoResearch Laboratories, Inc.

West Grove, PA 19390

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

1-800-367-5296

Catalog # 011-000-001

### Secondary Antibody: Donkey Anti-Rabbit IgG (H+L) Biotin-SP-Conjugated

Jackson ImmunoResearch Laboratories, Inc.

West Grove, PA 19390

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

1-800-367-5296

Catalog # 711-065-152

Label Complex: Vectastain Elite ABC Kit (Standard)

Vector Laboratories, Inc.

Burlingame, CA 94010

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

1-800-227-6666

Catalog # PK-6100

**Staining Procedure**

Positive Control Tissue: Red blood cells

Stain Localization: Membrane and 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 slides in 2 changes of 1X Wash Buffer for 5 minutes each.

4. Block with 10% Normal Donkey 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 1:10 dilution, and incubate for 1 hour at room temperature.

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

For negative control slides, dilute the protein concentration of the normal rabbit serum to match the protein concentration of the primary antibody. Make a 1:10 dilution from this normalized serum, and apply to the slides. Incubate for 1 hour at room temperature.

Lot # \_\_\_\_\_ Date Reconstituted \_\_\_\_\_

7. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.
8. Apply the donkey anti-rabbit secondary antibody at a 1:500 dilution, and incubate for 30 minutes at room temperature.  
Lot # \_\_\_\_\_ Date Reconstituted \_\_\_\_\_
9. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.
10. Apply the label complex from the Standard Elite Kit, and incubate for 30 minutes at room temperature. (Prepare at least 30 minutes prior to use.)  
Exp. Date \_\_\_\_\_ New Kit: yes / no
11. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each time.
12. 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
13. Rinse the slides in tap water 3 minutes.
14. Counterstain with Harris Hematoxylin for 20 seconds.
15. Rinse the slides in tap water until water is clear.
16. Gently agitate slides in 1X Wash Buffer until they turn blue.
17. 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

18. Coverslip

*Updated 03/18/09*