Detection of Hemoglobin in Formalin-Fixed, Paraffin-Embedded Rat Tissue

Reagent and Antibody Information

1X Wash Buffer
3% Hydrogen Peroxide
1% BSA Diluent
Normal Rabbit IgG – Affinity Purified
DAB Chromogen
Hematoxylin

Blocking Serum: Normal Donkey Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog # 017-000-011

Avidin / Biotin Blocking Kit
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog # SP-2001

Primary Antibody: Rabbit Polyclonal Hemoglobin β/γ/δ Antibody (H-76)
Santa Cruz Biotechnology
Santa Cruz, CA 95060
www.scbt.com
1-800-457-3801
Catalog # sc-21006

Secondary Antibody: Donkey Anti-Rabbit IgG (H+L) Biotin-SP-Conjugated
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog # 711-065-152

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: Red blood cells
Stain Localization: Membrane and cytoplasmic

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 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. Incubate for 1 hour 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 1:10 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 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 donkey anti-rabbit secondary antibody at a 1:500 dilution. 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 Vectastain R.T.U. Elite Label and incubate for 30 minutes at room temperature.
    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 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

13. Rinse the slides in tap water 3 minutes.

14. Counterstain with hematoxylin for 20 seconds.

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

16. Gently agitate slides in 1X wash buffer until the tissues 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