

Detection of BRDU in Formalin-Fixed, Paraffin Embedded Rodent Tissue

Reagents:

[1X Automation Buffer](#)
[3% Hydrogen Peroxide](#)
[Antibody Diluent](#)
[2N Hydrochloric Acid](#)
[Boric Acid-Borate Buffer](#)
[0.05M Tris-HCl](#)
[DAB Chromagen](#)
[Hematoxylin](#)

Antibody Information

Avidin Biotin Blocking Kit

Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog: #SP-2001

Kit: Vector Elite Rat IgG ABC Kit

Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog # PK 6104

*The Vector Rat Elite Kit contains solutions needed to make the secondary and label antibodies.

Primary antibody: Rat anti-BRDU

Accurate Chemical and Scientific Corp.
Westbury, NY 11590
Catalog #OBT0030

Staining Procedure

-Positive Control Tissue: small intestine.

-Stain localization: Nuclear

Deparaffinize and hydrate slides through the following solutions.

Xylene	2 times	5 minutes
100% EtOH	2 times	3 minutes
95% EtOH	2 times	3 minutes
1X Automation Buffer	2 times	5 minutes

1. Place slides in 2N Hydrochloric Acid at 37 °C for 30 minutes in a water bath.
 2. Place slides in Boric Acid-Borate Buffer for 1 minute at room temperature.
 3. Incubate the slides in 0.01% trypsin and incubate at 37°C for 3 minutes.
(DO NOT add the trypsin to the 0.05M Tris-HCl solution until 5 minutes prior to incubation. Trypsin loses 75% of its reactivity within 30 minutes at 37°C.)
Lot# _____ Exp Date _____
 4. Rinse in distilled water for 1 minute to stop the digestion.
 5. Rinse in 2 changes of 1X Automation buffer for 5 minutes each.
 6. Quench endogenous peroxidase by placing slides in 3% hydrogen peroxide for 10 minutes.
 7. Rinse in 2 changes of 1X Automation buffer for 5 minutes each.
 8. Apply block from Vector Rat Elite Kit and incubate for 20 minutes at room temperature.
Exp Date _____ New Kit: yes / no
- DO NOT RINSE SLIDES. CONTINUE TO AVIDIN-BIOTIN BLOCK.
9. Apply Avidin/Biotin block
Kit Lot# _____ Exp. Date _____ New Kit: yes / no
Apply avidin block - 15 min at RT.

Quick rinse in 1X AB.
Apply biotin block - 15 min at RT.

DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY

10. Apply primary antibody at a 1:800 dilution and incubate 30 minutes at room temperature. (This dilution is good for lot no. C90K250.)
Lot# _____ Aliquoted yes / no Aliquoted date _____

11. Rinse in 2 changes of 1X Automation buffer for 5 minutes each.

12. Apply secondary antibody from Vector Rat Elite Kit and incubate for 30 minutes at room temperature.

13. Rinse in 2 changes of 1X Automation buffer for 5 minutes each.

14. Apply Label antibody from Vector Rat Elite Kit and incubate for 30 minutes at room temperature. (Prepare 30 minutes prior to use)

15. Rinse in 2 changes of 1X Automation buffer for 5 minutes each.

16. Apply liquid Dako DAB Chromagen for 6 minutes in the dark.
(Add 1 drop of DAB per ml of substrate)
Lot# _____ Exp Date _____ New Kit: yes / no

17. Rinse in tap water 3 minutes.

18. Counterstain with Modified Harris Hematoxylin for 2 minutes.

19. Rinse in tap water until water is clear.

20. Place slides in 1X Automation buffer for 1 minute with gentle agitation to blue slides.

21. Dehydrate through the following solutions.

95% ETOH	1 changes	3 minutes
100% ETOH	3 changes	3 minutes
xylene	2 changes	5 minutes

22. Coverslip

updated 09/07/05