

Detection of BRDU in Formalin-Fixed, Paraffin Embedded Mouse 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 used: Vector Rat IgG Standard Kit

Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog: PK-4004

*The Vector Rat IgG Standard Kit includes reagents needed to make blocking solution, 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 blocking serum from Vector standard kit. 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 @ RT.

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

DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY

10. Apply primary antibody and incubate 30 minutes at room temperature at 1:400 dilution
Lot# _____ Aliquoted yes / no Aliquoted date _____

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

12. Apply the secondary antibody from Vector standard kit and incubate 20 minutes at room temperature.

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

14. Apply the label antibody from Vector standard kit and incubate 20 minutes at room temperature. (Prepare label at least 30min before incubation)

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 03/18/04