Detection of CYP2B1/2 in Formalin-Fixed, Paraffin-Embedded Rat Tissue

Reagents:
1X Automation Buffer
3% Hydrogen Peroxide
Antibody Diluent
Citrate Buffer
DAB Chromagen
Hematoxylin

Antibody Information:

Block: Protein Block Serum-Free Ready-To-Use
Dakocytomation USA
Carpinteria CA 93013
www.dakousa.com
1-800-235-5763
Catalog #X0909

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

Primary antibody: Rabbit anti-Human Cytochrome P450 CYP2B6 and Rat CYP2B1/2
Chemicon International, Inc
Temecula, CA 92590
www.chemicon.com
1-800-437-7500
Catalog #AB1283

Negative control serum: Normal Rabbit Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog #011-000-001

LSAB+ System-HRP
Dakocytomation USA
Carpinteria CA 93013
www.dakousa.com
Catalog #K0690
* This kit contains all the reagents necessary for secondary and label antibodies.

Staining Procedure

-Positive Control Tissue: Rat Liver
-Stain Localization: Cytoplasmic

Deparaffinize and hydrate slides through the following solutions:

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

1. Quench endogenous peroxidase by placing slides in 3% hydrogen peroxide for 15 minutes.

2. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

3. Perform Heat Induced Epitope Retrieval using a Microwave Oven
Unmasking Techniques
Place a full rack of slides in a Tissue Tek™ container containing 250ml of citrate buffer.
Microwave for 5 minutes at power level 5.
Cool for 1 minute (Add 50ml of citrate buffer to the container, if necessary).
Microwave again for 5 minutes at power level 5.
Remove the slides from the microwave oven and cool 20 minutes at room temperature.
Rinse slides in 2 changes of distilled water for 3 minutes each.

4. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

5. Incubate slides in Dako Serum-Free Protein Block for 10 minutes at room temperature.
Lot#_____ Exp. Date_____

6. Apply Avidin/Biotin block
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.
Wipe excess block

DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.

7. Apply primary antibody (Cyp2B1/2) at a 1:500 dilution and incubate for 30 minutes at room temperature.
Lot#_____ Aliquoted yes / no Date Aliquoted_____

For negative control slides, normalize the normal rabbit serum to the protein concentration of the primary antibody (Cyp2B1/2) and use this to make a 1:500 dilution. Apply to slides and incubate for 30 minutes at room temperature.
Lot#_____ Reconstituted Date _____

8. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

LSAB+ Kit Lot#_____ Exp. Date_____

9. Apply Link – Secondary (yellow bottle) from LSAB+ Kit and incubate for 30 minutes at room temperature.

10. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

11. Apply Label (red bottle) from LSAB+ Kit and incubate for 30 minutes at room temperature.

12. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

13. 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

14. Rinse in tap water 3 minutes.

15. Counterstain with Modified Harris Hematoxylin for 30 seconds.

16. Rinse in tap water until water is clear.

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

18. Dehydrate through the following solutions.

95% Ethanol	1 change	3 minutes
100% Ethanol	3 changes	3 minutes
Xylene	2 changes	5 minutes

19. Coverslip

updated 02/01/06