

Detection of CYP4A in Formalin-Fixed, Paraffin Embedded Mouse Tissue

Reagents:

[1X Automation Buffer](#)
[3% Hydrogen Peroxide](#)
[Antibody Diluent](#)
[DAB Chromagen](#)
[Hematoxylin](#)

Antibody Information:

Normal Rabbit Serum

Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog #011-000-001

Avidin Biotin Blocking Kit

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

Primary antibody: Sheep anti-rat CYP4A

Amersham Life Science
Piscataway, NJ 08855
www.apbiotech.com
1-800-526-3593
Western Blotting Kit
Catalog #RPN260

Negative control serum: Normal Sheep Serum

Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog #013-000-001

Secondary antibody: Biotinylated Rabbit anti-Sheep

Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390

www.jacksonimmuno.com

1-800-367-5296

Catalog #313-065-003

Label antibody: Vector EliteVectastain® ABC

Vector Laboratories, Inc.

Burlingame, CA 94010

www.vectorlabs.com

1-800-227-6666

Catalog #PK-6100

Staining Procedure

-Positive Control Tissue: Clofibrate Treated Mouse Liver

-Stain localization: Cytoplasmic

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. 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. No Retrieval

4. Block using 5% Normal Rabbit Serum for 20 minutes.

Lot# _____ Reconstituted Date _____

5. 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.

No wash, wipe excess block and apply primary antibody

6. Apply primary antibody CYP4A at a 1:250 dilution and incubate for 1 hour.

Lot# _____ Exp Date _____

On negative control slides, normalize the protein concentration of the normal sheep serum with protein concentration of the primary antibody (CYP4A) and use this to make the 1:250 dilution. Apply to slides and incubate for one hour.

Lot# _____ Reconstituted Date _____

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

8. Apply secondary antibody from Vector Rabbit anti-sheep at a 1:500 dilution and incubate for 30 minutes.

Lot # _____ Reconstituted Date _____

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

10. Apply Label antibody from Vector Elite Kit and incubate for 30 minutes.
(Prepare 30 minutes prior to use)

Exp. Date _____ New Kit: yes / no

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

12. 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

13. Rinse in tap water 3 minutes.

14. Counterstain with Modified Harris Hematoxylin for 30 seconds.

15. Rinse in tap water until water is clear.

16. Place slides in 1X Automation Buffer for 1 minute with gentle agitation to blue slides.

17. Dehydrate through the following solutions.

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

18. Coverslip

updated 01/14/2004