

# Detection of CYP4A in Formalin-Fixed, Paraffin Embedded Rat Tissue

Reagents:

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

## Antibody Information:

Blocking Serum: Normal Rabbit Serum  
Jackson Immunoresearch Laboratories, Inc.  
West Grove, PA 19390  
[www.jacksonimmuno.com](http://www.jacksonimmuno.com)  
1-800-367-5296  
Catalog #011-000-001

Avidin Biotin Blocking Kit  
Vector Laboratories, Inc.  
Burlingame, CA 94010  
[www.vectorlabs.com](http://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](http://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](http://www.jacksonimmuno.com)  
1-800-367-5296  
Catalog #013-000-001

Secondary antibody: Rabbit anti-Sheep  
Jackson Immunoresearch Laboratories, Inc.  
West Grove, PA 19390  
[www.jacksonimmuno.com](http://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](http://www.vectorlabs.com)  
1-800-227-6666  
Catalog #PK-6100

### Staining Procedure

-Positive Control Tissue: Peroxisome Study Rat 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. Block using 5% Normal Rabbit Serum for 20 minutes.

Lot# \_\_\_\_\_ Reconstituted Date \_\_\_\_\_

4. Apply Avidin/Biotin block

Lot # \_\_\_\_\_ Exp Date \_\_\_\_\_ New Kit      yes      /      no

Apply avidin block - 15 min @ RT.

Quick rinse in 1X AB.

Apply biotin block - 15 min @ RT.

No wash, wipe excess block and apply primary antibody

5. Apply primary antibody CYP4A at a 1:400 dilution and incubate for 1 hour.

Lot# \_\_\_\_\_ Aliquoted yes / no      Date Aliquoted \_\_\_\_\_

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 1:400 dilution. Apply to slides and incubate for one hour.  
Lot#\_\_\_\_\_ Reconstituted Date\_\_\_\_\_

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

7. Apply secondary antibody from Vector Rabbit anti-sheep at a 1:500 dilution and incubate for 30 minutes.  
Lot#\_\_\_\_\_ Reconstituted Date\_\_\_\_\_

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

9. Apply Label antibody from Vector Elite Kit and incubate for 30 minutes.  
(Prepare 30 minutes prior to use)  
Exp. Date \_\_\_\_\_ New Kit yes / no

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

11. 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

12. Rinse in tap water 3 minutes

13. Counterstain with Modified Harris Hematoxylin for 30 seconds.

14. Rinse in tap water until water is clear.

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

16. Dehydrate through the following solutions.

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

17. Coverslip

updated 01/14/2004