

Detection of CYP1A2 in Formalin-Fixed, Paraffin-Embedded Rat Tissue

Reagent and Antibody Information

[1X Wash Buffer](#)

[3% Hydrogen Peroxide](#)

[1% BSA Diluent](#)

[1X Citrate Buffer](#)

[DAB Chromagen](#)

[Hematoxylin](#)

Blocking Serum: 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 Cytochrome P450 CYP1A2

Chemicon International, Inc

Temecula, CA 92590

www.chemicon.com

1-800-437-7500

Catalog # AB1249

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: Rabbit Anti-Sheep IgG (H+L) Biotin-SP-Conjugated

Jackson Immunoresearch Laboratories, Inc.

West Grove, PA 19390

www.jacksonimmuno.com

1-800-367-5296

Catalog # 313-065-003

Label Complex: Peroxidase-Conjugated Streptavidin SS Label

Biogenex Laboratories
San Ramon, CA 94583
www.biogenex.com
1-800-421-4149
Catalog # HK330-9K

Staining Procedure

Positive Control Tissue: Liver (upregulated by treatment)
Stain Localization: Cytoplasmic (centrilobular staining pattern)

1. 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 Wash Buffer	2 times	5 minutes

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

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

4. Heat-Induced Epitope Retrieval Using The Microwave

Place a full rack of slides into a Tissue Tek® container with 200 ml of 1X citrate buffer
(Insert blank slides into any empty slots in the rack to ensure even heating of slides)

Microwave for 5 minutes at power level 5.

Cool for 1 minute. (Add more citrate buffer, if necessary.)

Microwave again for 5 minutes at power level 5. *Temperature Before Cooling Slides* _____

Cool 20 minutes at room temperature.

Rinse the slides in 2 changes of distilled water for 3 minutes each time.

6. Block with 10% Normal Rabbit Serum for 20 minutes at room temperature.

Lot # _____ Date Reconstituted _____

DO NOT RINSE SLIDES. CONTINUE TO AVIDIN-BIOTIN BLOCK.

7. Avidin / Biotin Blocking Kit

Lot # _____ Exp. Date _____ New Kit: yes / no

Apply avidin block - 15 minutes at room temperature.

Quick rinse in 1X Wash Buffer.

Apply biotin block - 15 minutes at room temperature.

DO NOT RINSE SECTIONS WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.
ONLY WIPE EXCESS BLOCK.

8. Apply primary antibody at a 1:400 dilution and incubate for 1 hour at room temperature.
Lot # _____ Exp. Date _____

For negative control slides, dilute the protein concentration of the normal sheep serum to match that of the primary antibody. Make a 1:400 dilution from this normalized serum and apply to the slides. Incubate for 1 hour at room temperature.
Lot # _____ Date Reconstituted _____

9. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each time.

10. Apply the rabbit anti-sheep secondary antibody at a 1:500 dilution, and incubate for 30 minutes at room temperature.
Lot # _____ Date Reconstituted _____

11. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each time.

12. Apply the Streptavidin SS Label, and incubate for 30 minutes at room temperature.
Lot # _____ Exp. Date _____

13. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.

14. Apply the DAB chromagen and incubate in the dark for 6 minutes at room temperature.
(Add 1 drop of DAB per ml of substrate)
Lot # _____ Exp. Date _____ New Kit: yes / no

15. Rinse the slides in tap water 3 minutes.

16. Counterstain with Harris Hematoxylin for 30 seconds.

17. Rinse the slides in tap water until water is clear.

18. Gently agitate slides in 1X Wash Buffer until they turn blue.

19. Dehydrate through the following solutions:

95% Ethanol	1 time	3 minutes
100% Ethanol	3 times	3 minutes
Xylene	2 times	5 minutes

20. Coverslip