

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

Reagent and Antibody Information

[1X Wash Buffer](#)

[3% Hydrogen Peroxide](#)

[1% BSA Diluent](#)

[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-Human Catalase

The Binding Site

Birmingham, England

Catalog # PC136

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 IgG (H+L)

Vector Laboratories, Inc.

Burlingame, CA 94010

www.vectorlabs.com

1-800-227-6666

Catalog # BA-6000

Label Complex: Vectastain Standard Elite ABC Kit

Vector Laboratories, Inc.

Burlingame, CA 94010

www.vectorlabs.com

1-800-227-6666

Catalog # PK-6100

Staining Procedure

Positive Control Tissue: Clofibrate-treated rat liver

Stain Localization: Cytoplasmic

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 slides in 3% hydrogen peroxide for 15 minutes.

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

4. Block with 5% Normal Rabbit Serum for 20 minutes at room temperature.

Lot # _____ Date Reconstituted _____

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

5. 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.

6. Apply primary antibody at a 1:500 dilution and incubate for 1 hour at room temperature.

Lot # _____ Date Aliquoted _____

For negative control slides, dilute the protein concentration of the normal sheep serum to that of the primary antibody. Make a 1:500 dilution from this normalized serum and apply to the slides. Incubate for 1 hour at room temperature.

Lot # _____ Date Reconstituted _____

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

8. Apply the rabbit anti-sheep secondary antibody at a 1:500 dilution and incubate for 30 minutes at room temperature.

Lot # _____ Reconstituted Date _____

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

10. Apply the label complex from the Standard Elite Kit and incubate for 30 minutes at room temperature. (Prepare at least 30 minutes prior to use.)

Exp. Date _____ New Kit: yes / no

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

12. Apply 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

13. Rinse the slides in tap water 3 minutes.

14. Counterstain with Harris Hematoxylin for 30 seconds.

15. Rinse in tap water until water is clear.

16. Gently agitate slides in 1X Wash buffer until they turn blue.

17. Dehydrate through the following solutions:

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

18. Coverslip

Updated 01/06/04