

# Detection of Superoxide Dismutase 2 in Formalin-Fixed, Paraffin-Embedded Rat Tissue

## Reagents

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

## Antibody Information

Kit: Rabbit IgG Elite Kit  
Vector Laboratories, Inc.  
Burlingame, CA 94010  
[www.vectorlabs.com](http://www.vectorlabs.com)  
1-800-227-6666  
Catalog # PK-6101

Note: The Vector Rabbit Elite Kit contains solutions needed to make the block, secondary and label antibodies.

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: Rabbit Polyclonal Antibody to Superoxide Dismutase 2  
Abcam Inc  
Cambridge, MA 02139  
[www.abcam.com](http://www.abcam.com)  
1-888-772-2226  
Catalog # ab13533-50

Negative control 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

## Staining Procedure

Positive Control Tissue: Normal rat brain or LPS-treated 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. Unmasking technique using the decloaker.  
Add 500 ml distilled water to the pan of the decloaker.  
Place full rack of slides in 200 ml of 1X citrate buffer and place in the decloaker.  
Decloak for 5 minutes. Pressure \_\_\_\_\_  
Depressurize for 10 minutes.  
Remove pan top and cool for 10 minutes. Temperature before cooling \_\_\_\_\_  
Rinse in distilled water twice for 3 minutes each.
4. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.
5. Apply blocking solution from the Vector Rabbit Elite Kit and incubate for 20 minutes at room temperature.  
Exp. Date \_\_\_\_\_ New Kit: yes / no

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

6. Apply the Avidin Biotin Blocking Kit  
Lot# \_\_\_\_\_ Exp Date \_\_\_\_\_ New Kit: yes / no  
Apply avidin block - 15 minutes at room temperature.  
Quick rinse in 1X Automation Buffer.  
Apply biotin block - 15 minutes at room temperature.  
No wash, wipe excess block and apply primary antibody

DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.

7. Apply primary antibody (SOD2) at a 1:1000 dilution and incubate for one hour at room temperature  
Lot# \_\_\_\_\_ Exp Date \_\_\_\_\_

For negative control slides, normalize the protein concentration of the normal rabbit serum to the protein concentration of the primary antibody (SOD2). Use this to make a 1:1000 dilution. Apply to slides and incubate for one hour at room temperature.  
Lot# \_\_\_\_\_ Reconstituted Date \_\_\_\_\_

8. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.
9. Apply secondary antibody from Vector Rabbit Elite 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 antibody from Vector Rabbit Elite 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 20 seconds.
16. Rinse in tap water until water is clear.
17. Gently agitate slides in 1X Automation buffer until they turn blue.
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 12/19/06*