

# Detection of Tyrosine Hydroxylase in Formalin Fixed, Paraffin-Embedded Mouse Tissue

## **Reagent and Antibody Information**

[1X Wash Buffer](#)

[3% Hydrogen Peroxide](#)

[1% BSA Diluent](#)

[Normal Rabbit IgG – Affinity Purified](#)

[DAB Chromogen](#)

[Hematoxylin](#)

Staining Kit: Vectastain Elite ABC Kit (Rabbit IgG)

Vector Laboratories, Inc.

Burlingame, CA 94010

[www.vectorlabs.com](http://www.vectorlabs.com)

1-800-227-6666

Catalog # PK-6101

**Note:** This kit contains all reagents necessary to make the blocking reagent, secondary antibody and label complex.

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 Anti-Tyrosine Hydroxylase

Millipore

Billerica, Massachusetts 01821

[www.millipore.com](http://www.millipore.com)

1-800-645-5476

Catalog # AB152

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: Brain (substantia nigra)

Stain Localization: Cytoplasmic

1. Deparaffinize and hydrate slides through the following solutions:

| Solution       | Repetitions | Time      |
|----------------|-------------|-----------|
| 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.

Vectastain Rabbit Elite Staining Kit

Exp Date \_\_\_\_\_ New Kit: yes / no

4. Apply the block from the Rabbit Elite Kit. Incubate for 20 minutes at room temperature.

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

5. Avidin / Biotin Blocking Kit

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

Apply avidin block for 15 minutes at room temperature.

Quick rinse in 1X wash buffer.

Apply biotin block for 15 minutes at room temperature.

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

6. Apply primary antibody at a 1:1000 dilution. Incubate for 1 hour at room temperature.

Lot # \_\_\_\_\_ Exp. Date \_\_\_\_\_

For negative control slides, dilute normal rabbit serum so that its protein concentration matches that of the primary antibody (if necessary). Then make a 1:1000 dilution. Apply the negative and 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 secondary antibody from Rabbit Elite Kit. Incubate for 30 minutes at room temperature.

9. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.

10. Apply the label complex from the Rabbit Elite Kit. Incubate for 30 minutes at room temperature.

(Prepare at least 30 minutes prior to use.)

11. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.
12. Apply the DAB chromogen. 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 hematoxylin for 20 seconds.
15. Rinse the slides in tap water until water is clear.
16. Gently agitate slides in 1X wash buffer until the tissues turn blue.
17. Dehydrate through the following solutions:

| Solutions    | Repetitions | Time      |
|--------------|-------------|-----------|
| 95% Ethanol  | 1 time      | 3 minutes |
| 100% Ethanol | 3 times     | 3 minutes |
| Xylene       | 2 times     | 5 minutes |

18. Coverslip

*Updated 10/31/08*