

# Detection of HDAC2 in Formalin-Fixed, Paraffin-Embedded Human Tissue

## Reagent and Antibody Information

[1X Wash Buffer](#)

[3% Hydrogen Peroxide](#)

[1% BSA Diluent](#)

[1X Citrate Buffer](#)

[DAB Chromogen](#)

[Hematoxylin](#)

### Staining Kit: Vectastain Elite ABC Kit (Mouse IgG)

Vector Laboratories, Inc.

Burlingame, CA 94010

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

1-800-227-6666

Catalog # PK-6102

**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: Mouse Anti-HDAC2 Monoclonal Antibody (C-8)

Santa Cruz Biotechnology, Inc.

Santa Cruz, CA 95060

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

1-800-457-3801

Catalog # sc-9959

### Negative Control Serum: Normal Mouse IgG

Santa Cruz Biotechnology, Inc.

Santa Cruz, CA 95060

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

1-800-457-3801

Catalog # sc-2025

## Staining Procedure

Positive Control Tissue: Breast carcinoma or teratoma  
Stain Localization: Nuclear

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

4. Heat-Induced Epitope Retrieval Using The Decloaker

Add 500 ml of distilled water to the pan inside the decloaker.

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)

Place the container stably inside the pan and decloak for 5 minutes. *Maximum Pressure* \_\_\_\_\_

Depressurize for 10 minutes.

Remove pan top and cool for 10 minutes. *Temperature Before Cooling Slides* \_\_\_\_\_

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

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

Vectastain Mouse Elite Staining Kit

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

6. Apply the block from the Mouse Elite Kit and incubate for 20 minutes at room temperature.

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

7. 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.

8. Apply primary antibody at a 1:10 dilution. Incubate for 1 hour at room temperature.

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

For negative control slides, dilute normal mouse IgG so that it's IgG protein concentration matches that of the primary antibody (if necessary). Then make a 1:10 dilution. If the concentrations can't be matched using this method, the dilution for the negative reagent may need to be adjusted. Apply the negative and incubate for 1 hour at room temperature.

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

9. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.
10. Apply the secondary antibody from Mouse Elite Kit. Incubate for 30 minutes at room temperature.
11. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.
12. Apply the label complex from the Mouse Elite Kit. Incubate for 30 minutes at room temperature. (Prepare at least 30 minutes prior to use.)
13. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.
14. 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
15. Rinse the slides in tap water 3 minutes.
16. Counterstain with hematoxylin for 20 seconds.
17. Rinse the slides in tap water until water is clear.
18. Gently agitate slides in 1X wash buffer until the tissues turn blue.
19. Dehydrate through the following solutions:

<b>Solutions</b>	<b>Repetitions</b>	<b>Time</b>
95% Ethanol	1 time	3 minutes
100% Ethanol	3 times	3 minutes
Xylene	2 times	5 minutes

20. Coverslip

*Updated 12/15/06*