

Detection of 5mCytidine in Formalin Fixed, Paraffin-Embedded Mouse Tissue

Reagents:

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

Antibody Information:

Kit: Rabbit Elite Kit

Vector Laboratories, Inc.
Burlingame, CA 94010

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
1-800-227-6666

Catalog #SP-2001

Primary antibody: Anti-5mCytidine Polyclonal Antibody

Megabase Research Products

Lincoln, NE 68504

<http://www.pcrjet.com/>
1-402-467-6499

Catalog # CP50250

Negative control serum: Normal Rabbit Serum

Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390

www.jacksonimmuno.com
1-800-367-5296

Catalog # 011-000-001

Staining Procedure

Positive Control Tissue: brain, lung, liver, pancreas, kidney

Stain Localization: Nuclear

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 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 500ml of distilled water to the pan of the decloaker.
Place a full rack of slides in a Tissue Tek™ container containing 250ml of 1X citrate buffer solution.
Decloak for 5 minutes. Pressure _____
Depressurize for 10 minutes.
Remove pan top and cool for 10 minutes. Temperature _____
Rinse in distilled water two times for 3 minutes each

4. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

Rabbit Vector Elite Kit: Lot# _____ exp _____

5. Apply blocking serum from Rabbit Elite Kit and incubate for 20 minutes at room temperature.

DO NOT RINSE SLIDES. CONTINUE TO AVIDIN-BIOTIN.

6. Apply Avidin/Biotin block
Lot# _____ Exp Date _____ New Kit: yes / no
Apply avidin block - 15 min at RT.
Quick rinse in 1X AB.
Apply biotin block - 15 min at RT.
Wipe excess block

DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.

7. Apply primary antibody (5mCytidine) at a 1:2000 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 (5mCytidine) and use this to make a 1:2000 dilution 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 the secondary antibody from the Vector Rabbit Elite Kit and incubate 30 minutes at room temperature.

10. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

11. Apply the label antibody from the Vector Rabbit Elite Kit and incubate 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 30 seconds.

16. Rinse in tap water until water is clear.

17. Place slides in 1X Automation buffer for 1 minute with gentle agitation to blue slides.

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 05/24/06