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

Reagent and Antibody Information

1X Wash Buffer
3% Hydrogen Peroxide
1% BSA Diluent
1X Citrate Buffer
DAB Chromagen
Hematoxylin

Staining Kit: Vectastain Elite ABC Kit (Rabbit IgG)
Vector Laboratories, Inc.
Burlingame, CA 94010
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
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

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 Rabbit Elite Staining Kit
   Exp Date_____ New Kit: yes / no

6. Apply the block from the Rabbit 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 BUFFER.

8. Apply primary antibody at a 1:2000 dilution, and incubate for 1 hour at room temperature.
   Lot #_____ Exp Date _____

For negative control slides, dilute the protein concentration of the normal rabbit serum to match that of the primary antibody. Make a 1:2000 dilution from this normalized serum, and apply to the slides. Incubate for 1 hour at room temperature.
Lot #_____ Date Reconstituted_____

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

10. Apply the secondary antibody from Rabbit Elite Kit, and 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 Rabbit Elite Kit, and 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 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

15. Rinse the slides in tap water 3 minutes.

16. Counterstain with Harris Hematoxylin for 20 seconds.

17. Rinse the slides in tap water until water is clear.

18. Gently agitate slides in 1X Wash Buffer until they turn blue.

19. Dehydrate through the following solutions:

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

20. Coverslip

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