Detection of Ki-67 (MIB 5) in Formalin Fixed Paraffin-Embedded Rat Tissue

Reagents:

1X Automation Buffer
3% Hydrogen Peroxide
Antibody Diluent
Citrate Buffer
DAB Chromagen
Hematoxylin

Antibody Information

Blocking Serum: Normal Horse Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog #008-000-001

Avidin/Biotin Blocking Kit
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog #SP-2001

Primary antibody: Mouse anti- rat Ki-67 (MIB 5)
Dako Corporation
Carpinteria, CA 93013
www.dakousa.com
1-800-235-5763
Catalog #M7248

Negative Control: Normal Mouse Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog #015-000-001

Secondary antibody: Biotinylated horse anti-mouse IgG
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog #BA-2001

Label antibody:Vector Standard Elite

Vector Laboratories, Inc.

Burlingame, CA 94010

www.vectorlabs.com

1-800-227-6666

Catalog #PK-6100

Staining Procedure

-Positive Control Tissue: Partial Hep Liver
-Stain Localization: Nuclear; localizes in the chromatin.

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 Techniques using the decloaker.
Add 500 ml D/W 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 min.Temp_____
Rinse in D/W, 2x for 3 min each

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

5. Apply 5% Normal Horse Serum solution for 20 minutes at room temperature.
Lot#_____ Reconstituted Date_____

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 SECTIONS WITH BUFFER.

7. Apply primary antibody (mouse anti-Ki-67) at a 1:100 dilution and incubate 1 hour.
Lot#_____ Exp. Date_____

For negative control slides, normalize the protein concentration of normal mouse serum to the protein concentration of the primary antibody (mouse Ki-67) and use this to make the 1:100 dilution. Apply to slides and Incubate for one hour.
Lot #_____ Reconstituted Date_____

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

9. Apply biotinylated horse anti-mouse secondary antibody at a 1:300 dilution and incubate for 30 minutes.
Lot#_____ Exp Date_____

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

11. Apply the label antibody and incubate for 30 minutes. (Prepare atleast 30mins before use)
Exp. Date_____ New Kit: yes / no

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.
Update 5/19/04