# Detection of Ki-67 (TEC-3) in Formalin Fixed Paraffin-Embedded Mouse Tissue

## Reagents:

1X Automation Buffer

3% Hydrogen Peroxide

Antibody Diluent

Citrate Buffer

DAB Chromagen

Hematoxylin

### Antibody Information

Block: Normal Rabbit Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog #011-000-001

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

Negative control: Normal Rat Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296

Primary antibody: Rat anti- mouse Ki-67 (TEC 3)
Dako Corporation
Carpinteria, CA 93013
www.dakousa.com
1-800-235-5763
Catalog #M7249

Secondary antibody: Biotinylated rabbit anti-rat IgG
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666

Label antibody :Vector Elite Label
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog #PK-6100

## Staining Procedure

-Positive Control Tissue: Mouse GI tissue
-Stain Localization: Restricted to the nucleus of proliferating cells with a distinct, prominent staining of nucleoli.

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 Rabbit 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.
No wash, wipe excess block
DO NOT RINSE SECTIONS WITH BUFFER.

7. Apply primary antibody (TEC-3 Ki-67) at 1:80 and incubate 1 hour.
Lot#_____ Exp Date_____

For negative control slides, normalize the protein concentration of normal rat serum to the protein concentration of the primary antibody and use this to make the 1:80 dilution. Apply normal rat serum to the 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 the Rabbit anti-rat secondary antibody at a 1:300 dilution and incubate for 30 minutes.
Lot#_____ Reconstituted Date_____

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

11. Apply the label antibody (Vector elite) and incubate for 30 minutes.
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.

last updated 10/12/04