

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

Reagent and Antibody Information

[1X Wash Buffer](#)

[3% Hydrogen Peroxide](#)

[1% BSA Diluent](#)

[1X Citrate Buffer](#)

[DAB Chromogen](#)

[Hematoxylin](#)

Blocking Solution: Dakocytomation Protein Block Serum-Free Ready-To-Use

Dakocytomation Corporation

Carpinteria, CA 93013

www.dako.com

1-800-235-5763

Code No. X0909

Avidin / Biotin Blocking Kit

Vector Laboratories, Inc.

Burlingame, CA 94010

www.vectorlabs.com

1-800-227-6666

Catalog # SP-2001

Primary Antibody: Monoclonal Mouse Anti-Rat Ki-67 Antibody (MIB-5)

Dakocytomation Corporation

Carpinteria, CA 93013

www.dako.com

1-800-235-5763

Code No. M7248

Secondary Antibody: Biotinylated Horse Anti-Mouse IgG (H+L)

Vector Laboratories, Inc.

Burlingame, CA 94010

www.vectorlabs.com

1-800-227-6666

Catalog # BA-2001

Label Complex: Peroxidase-Conjugated Streptavidin SS Label

Biogenex Laboratories

San Ramon, CA 94583

www.biogenex.com

1-800-421-4149

Catalog # HK330-9K

Staining Procedure

Positive Control Tissue: Gastrointestinal tract

Stain Localization: Nuclear (localizes in the chromatin)

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 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.

6. Block with the Dako protein-blocking reagent for 15 minutes at room temperature.

Lot # _____ Exp. Date _____

DO NOT RINSE 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 SECTIONS WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.

ONLY WIPE EXCESS BLOCK.

8. Apply primary antibody at a 1:100 dilution. Incubate for 1 hour at room temperature. (We typically do not run negatives with this stain.)

Lot # _____ Exp. Date _____

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

10. Apply the horse anti-mouse secondary antibody at a 1:100 dilution. Incubate for 30 minutes at room

temperature.

Lot # _____ Date Reconstituted _____

11. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.
12. Apply the Streptavidin SS Label. Incubate for 30 minutes at room temperature.
Lot # _____ Exp. Date _____
13. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.
14. Apply the DAB chromogen 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 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:

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

20. Coverslip

Update 06/11/08