

Detection of Ki-67 in Formalin-Fixed, Paraffin-Embedded Mouse Tissue

Reagent and Antibody Information

[1X Wash Buffer](#)

[3% Hydrogen Peroxide](#)

[1% BSA Diluent](#)

[1X Citrate Buffer](#)

[Normal Rabbit IgG – Affinity Purified](#)

[DAB Chromogen](#)

[Hematoxylin](#)

Blocking Solution: Rodent Block M

Biocare Medical

Concord, CA 94520

www.biocare.net

1-800-799-9499

Catalog # RBM961

Primary Antibody: Rabbit Polyclonal Anti-Ki-67 Antibody (SP6)

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Catalog # CRM325

Polymer Reagent: Rabbit-on-Rodent HRP-Polymer Detection

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1-800-799-9499

Catalog # RMR622

Staining Procedure

Positive Control Tissue: Gastrointestinal tract

Stain Localization: Nuclear (proliferating cells, distinctly stains nucleoli)

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. Heat-Induced Epitope Retrieval Using The Decloaker

Add 500 ml of distilled water to the pan inside the decloaker. All three of the decloaker's containers must be filled. Any containers without samples should have 250 ml of distilled water. The samples need to be in a container with a full rack of slides and about 200 ml of **1X citrate buffer**. (Insert blank slides into any empty slots in the rack to ensure even heating of slides.)

Decloak the slides for 15 minutes at 110°C. *Maximum Pressure* _____

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.

3. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.
4. Quench endogenous peroxidase by placing the slides in 3% hydrogen peroxide for 15 minutes.
5. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.
6. Block with the Rodent Block M for 20 minutes at room temperature.
Lot # _____ Exp. Date _____

DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.
ONLY WIPE EXCESS BLOCK.

7. Apply primary antibody at a 1:100 dilution. Incubate for 30 minutes at room temperature.
Lot # _____ Exp. Date _____
8. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.
9. Apply the Rabbit-on-Rodent HRP-Polymer Reagent, and incubate for 30 minutes at room temperature.
Lot # _____ Exp. Date _____

10. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.
11. Apply the DAB chromogen. 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
12. Rinse the slides in tap water 3 minutes.
13. Counterstain with hematoxylin for 20 seconds.
14. Rinse the slides in tap water until water is clear.
15. Gently agitate slides in 1X wash buffer for 20 seconds.
16. 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

17. Coverslip

Updated 09/23/15