

Detection of PCNA in Formalin-Fixed, Paraffin Embedded Mouse and Rat Tissue

Reagent and Antibody Information

[1X Wash Buffer](#)

[3% Hydrogen Peroxide](#)

[1% BSA Diluent](#)

Distilled Water

[DAB Chromogen](#)

[Hematoxylin](#)

[1% Dry Milk](#)

Primary Antibody: Mouse Monoclonal Anti-PCNA Antibody

Millipore

Billerica, Massachusetts 01821

www.millipore.com

1-800-645-5476

Catalog # MAB4078

Lot # 1981554

Secondary Antibody: Biotin-SP-conjugated AffiniPure Goat anti-Mouse IgM

Jackson ImmunoResearch Laboratories, Inc.

West Grove, PA 19390

www.jacksonimmuno.com

1-800-367-5296

Catalog # 115-065-020

Label Complex: R.T.U. Vectastain Elite ABC Reagent

Vector Laboratories, Inc.

Burlingame, CA 94010

www.vectorlabs.com

1-800-227-6666

Catalog # PK-7100

Staining Procedure

Positive Control Tissue: Gastrointestinal tract (any tissue with a high cell turnover rate)

Stain Localization: The localization of the stain is dependent upon the cell cycle stage. (Foley, J et al.)

G0 = no staining G1 = nuclear staining, 1+ just above background

S = nuclear, intense, dark brown staining G2 = nuclear and cytoplasmic, 2+ distinct brown staining

M = cytoplasmic, 2+ distinct granular brown staining

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 Microwave

Place a full rack of slides into a Tissue Tek® container with 200 ml of distilled water

(Insert blank slides into any empty slots in the rack to ensure even heating of slides)

Microwave for 5 minutes at power level 5.

Cool for 1 minute. (Add more distilled water, if necessary.)

Microwave again for 5 minutes at power level 5. *Temperature Before Cooling Slides* _____

Cool 20 minutes at room temperature.

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.

The diluent for the primary antibody will consist of a 1:1 mixture of 1% BSA diluent and 1% milk. The 1% milk should be prepared in distilled water.

6. Apply primary antibody at 1:1500 dilutions. Incubate for 1 hour at room temperature. (We typically do not run negatives with this stain.)

Lot # _____ Exp. Date _____

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

8. Apply the goat anti-mouse IgM secondary antibody at a 1:500 dilution. Incubate for 30 minutes at room temperature.

Lot # _____ Date Reconstituted _____

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

10. Apply the Vectastain R.T.U. Elite Label and incubate for 30 minutes at room temperature.

Exp. Date _____ New Kit: yes / no

11. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.
12. 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
13. Rinse the slides in tap water 3 minutes.
14. Counterstain with hematoxylin for 20 seconds.
15. Rinse the slides in tap water until water is clear.
16. Gently agitate slides in 1X wash buffer until the tissues turn blue.
17. 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

18. Coverslip

Updated 06/21/13