Detection of PCNA in Formalin-Fixed, Paraffin-Embedded Human Tissue

Reagent and Antibody Information

1X Wash Buffer
3% Hydrogen Peroxide
1% BSA Diluent
Distilled Water
DAB Chromagen
Hematoxylin
1% Dry Milk

Blocking Solution: 1% non-fat dry milk is used to dilute the primary antibody. This serves as the blocking solution for this protocol.

Primary Antibody: Monoclonal Anti-Mouse PCNA (19A2) Antibody
Chemicon International, Inc.
Temecula, CA 92590
www.chemicon.com
1-800-437-7500
Catalog # MAB4078

Secondary Antibody: Goat Anti-Mouse IgM mu (Chain-Specific Biotin Conjugated)
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog # 115-065-020

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: Tissue with a high cell turnover rate (breast carcinoma)
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 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, 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.

6. Prepare the primary antibody at a 1:1200 dilution, and incubate for 30 minutes at room temperature.
   (The diluent should consist of equal amounts of 1% non-fat dry milk and 1% BSA diluent.)
   Lot #_____ Date Aliquoted_____

7. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each time.

8. Apply the goat anti-mouse IgM secondary antibody at a 1:400 dilution, and incubate for 30 minutes at room temperature. (This reagent is made in BSA diluent only.)
   Lot #_____ Date Reconstituted_____

9. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.

10. Apply the Streptavidin SS Label and incubate for 30 minutes at room temperature.
    Lot #_____ Exp Date _____

11. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.

12. Apply the DAB chromagen, 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

13. Rinse the slides in tap water 3 minutes.

14. Counterstain with Harris Hematoxylin for 20 seconds.

15. Rinse the slides in tap water until water is clear.

16. Gently agitate slides in 1X Wash Buffer until they turn blue.

17. Dehydrate through the following solutions:

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

18. Coverslip

Updated 02/07/06