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

Reagents:
1X Automation Buffer
3% Hydrogen Peroxide
Antibody Diluent
DAB Chromagen
Hematoxylin
1% Dry Milk prepare in distilled water.

Antibody Information:

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

Primary antibody: Monoclonal antibody anti-mouse PCNA (19A2)
Chemicon International, Inc.
Temecula, CA 92590
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
Suggested dilution: 1:400

Label antibody: StriAviGen Super Sensitive Predilute Label Antibody
Biogenex Laboratories
San Ramon, CA 94583
www.biogenex.com
1-800-421-4149
Catalog #HK330-5K

Staining Procedure

-Positive Control Tissue: Tissue with a high rate of cell turnover (ie. small intestine, testis)
-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

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. Perform Heat Induced Epitope Retrieval using a microwave oven
Unmasking Techniques
Place a full rack of slides in a container containing 200ml of distilled water.
Microwave for 5 minutes at level 5.
Cool for 1 minute
Microwave again for 5 minutes at level 5. Temp._____
Remove the slides from the microwave oven and allow to cool 20 minutes at room temperature.
Rinse slides in distilled water for 2 minutes. Repeat two times.

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

5. Prepare primary antibody concentration at a 1:1500 dilution using equal amounts of 1% non-fat dry milk and diluent. Apply primary antibody and incubate for 30 minutes.
Lot #_____ Aliquoted yes / no Date Aliquoted_____

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

7. Prepare secondary antibody (Goat anti-mouse IgM mu chain-specific biotin conjugated) in diluent at a 1:400 dilution and incubate for 30 minutes at room temperature.
Lot#_____ Exp. Date_____

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

9. Apply Label (Biogenex) antibody and incubate for 30 minutes at room temperature.
Lot# _____ Exp. Date _____

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

11. Apply liquid DAB Chromagen for 6 minutes in the dark.
Lot# _____Exp. Date_____ New Kit: yes / no

12. Rinse in tap water 3 minutes.

13. Counterstain with Modified Harris Hematoxylin for 30 seconds.

14. Rinse in tap water until water is clear.

15. Place slides in 1X Automation buffer for 1 minute with gentle agitation to blue slides.

16. Dehydrate through the following solutions.

95% Ethanol	1 change	3 minutes
100% EtOH	3 changes	3 minutes
Xylene	2 changes	5 minutes

17. Coverslip

updated 02/07/06