Detection of Histone H2A.X in Formalin-Fixed, Paraffin-Embedded MouseTissue

Reagents:

1X Automation Buffer
3% Hydrogen Peroxide
Antibody Diluent
Citrate Buffer
DAB Chromagen
Hematoxylin

Antibody Information:

Block: Dako Protein Block Serum-Free
DAKO Corporation
Carpinteria CA 93013
www.dakousa.com
Catalog# X0909

Avidin/Biotin Blocking Kit
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog #SP-2001

Primary antibody: Rabbit anti-phoso-Histone H2A.X
Upstate Cell Signaling Solutions
www.upstate.com
1-800-233-3991
Catalog No. # 07-164

Negative control: Normal Rabbit Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296

Kit: DAKO LSAB+ System HRP
DAKO Corporation
Carpinteria CA 93013
www.dakousa.com
Code No. K06901

Staining Procedure

-Positive Control Tissue: Testis

-Stain Localization: Nuclear

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. Unmasking Techniques using the decloaker.
Add 500 ml D/W to the pan of the decloaker.
Place full rack of slides in 200 ml of 1X citrate buffer and place in the decloaker.
Decloak for 5 minutes. Pressure_____
Depressurize for 10 minutes.
Remove pan top and cool for 10 min.Temp_____
Rinse in D/W, 2x for 3 min each

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

5. Use serum-free All -Protein Dako for 10 mins
Lot#_____ Exp Date_____

DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.

6. Apply primary antibody (H2A.X) at a 1:250 dilution and incubate for one hour.
Lot#_____ Exp Date_____

For negative control slides, normalize the protein concentration of normal rabbit serum to the protein concentration of the complementary primary antibody and use this to make a 1:250 dilutions. Apply normal goat serum to the slides and incubate for one hour.
Lot#_____ Reconstituted Date_____

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

LSAB Kit Lot#_____ Exp Date_____

8. Apply Link – Secondary (yellow bottle) from LSAB+ Kit and incubate for 10 minutes.

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

10. Apply label (red bottle) from LSAB+ kit and incubate for 10 minutes.

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

12. Apply liquid Dako DAB Chromagen for 6 minutes in the dark.
(Add 1 drop of DAB per ml of substrate)
Lot #_____ Exp Date_____ New Kit: Yes / No

13. Rinse in tap water 3 minutes.

14. Counterstain with Modified Harris Hematoxylin for 30 seconds.

15. Rinse in tap water until water is clear.

16.Rinse slides in 1X automation buffer for 1 min with gentle agitation to blue slides.

17. Dehydrate through the following solutions.

95% EtOH	1 times	3 minutes
100% EtOH	3 times	3 minutes
Xylene	2 times	5 minutes

18. Coverslip
update 3/2/04