

# Detection of Histone H2A.X in Formalin-Fixed, Paraffin-Embedded Mouse Tissue

## Reagent and Antibody Information

[1X Wash Buffer](#)  
[3% Hydrogen Peroxide](#)  
[1% BSA Diluent](#)  
[1X Citrate Buffer](#)  
[DAB Chromagen](#)  
[Hematoxylin](#)

Blocking Solution: Dakocytomation Protein Block Serum-Free Ready-To-Use  
Dakocytomation Corporation  
Carpinteria CA 93013  
[www.dakousa.com](http://www.dakousa.com)  
1-800-235-5763  
Code No. X0909

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

Primary Antibody: Rabbit Anti-Phoso-Histone H2A.X Antibody  
Upstate Cell Signaling Solutions  
Charlottesville, VA 22903  
[www.upstate.com](http://www.upstate.com)  
1-800-233-3991  
Catalog # 07-164

Negative Control Serum: Normal Rabbit Serum  
Jackson Immunoresearch Laboratories, Inc.  
West Grove, PA 19390  
[www.jacksonimmuno.com](http://www.jacksonimmuno.com)  
1-800-367-5296  
Catalog # 011-000-001

Staining Kit: LSAB+ System-HRP  
Dakocytomation Corporation  
Carpinteria CA 93013  
[www.dakousa.com](http://www.dakousa.com)  
1-800-235-5763  
Code No. K0690

**Note:** This kit includes reagents needed for the secondary antibody (link) and label complex.

## Staining Procedure

Positive Control Tissue: Testis  
Stain Localization: Nuclear

1. Deparaffinize and hydrate slides through the following solutions:

<b>Solution</b>	<b>Repetitions</b>	<b>Time</b>
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 Decloaker

Add 500 ml of distilled water to the pan inside the decloaker.

Place a full rack of slides into a Tissue Tek® container with 200 ml of 1X citrate buffer

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

Place the container stably inside the pan and decloak for 5 minutes. *Maximum Pressure* \_\_\_\_\_

Depressurize for 10 minutes.

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.

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

6. Block with the Dako Protein Blocking Reagent for 10 minutes at room temperature.

Lot # \_\_\_\_\_ Exp Date \_\_\_\_\_

DO NOT RINSE THE SLIDES. CONTINUE TO AVIDIN-BIOTIN BLOCK.

7. Avidin / Biotin Blocking Kit

Lot # \_\_\_\_\_ Exp Date \_\_\_\_\_ New Kit: yes / no

Apply avidin block for 15 minutes at room temperature.

Quick rinse in 1X Wash Buffer.

Apply biotin block for 15 minutes at room temperature.

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

8. Apply the primary antibody at a 1:250 dilution, and incubate for 1 hour at room temperature.

Lot # \_\_\_\_\_ Date Aliquoted \_\_\_\_\_

For negative control slides, dilute the protein concentration of the normal rabbit serum to match that of

the primary antibody. Make a 1:250 dilution from this normalized serum, and apply to the slides. Incubate for 1 hour at room temperature.

Lot # \_\_\_\_\_ Date Reconstituted \_\_\_\_\_

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

LSAB+ Kit

Lot # \_\_\_\_\_ Exp Date \_\_\_\_\_

10. Apply the Link (yellow bottle) from the LSAB+ Kit, and incubate for 10 minutes at room temperature.

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

12. Apply the Label (red bottle) from the LSAB+ Kit, and incubate for 10 minutes at room temperature.

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

14. 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

15. Rinse the slides in tap water 3 minutes.

16. Counterstain with Harris Hematoxylin for 20 seconds.

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

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

19. Dehydrate through the following solutions:

<b>Solution</b>	<b>Repetitions</b>	<b>Time</b>
95% Ethanol	1 time	3 minutes
100% Ethanol	3 times	3 minutes
Xylene	2 times	5 minutes

20. Coverslip

*Updated 3/2/04*