

Detection of phospho-Histone H2B (Ser14) in Formalin-Fixed Paraffin-Embedded Rat Tissue

Reagents:

[1X Automation Buffer](#)

[3% Hydrogen Peroxide](#)

[Antibody Diluent](#)

[Citrate Buffer](#)

[DAB Chromagen](#)

[Hematoxylin](#)

Antibody Information

Blocking serum: Normal Donkey Serum

Jackson Immunoresearch Laboratories, Inc.

West Grove, PA 19390

www.jacksonimmuno.com

1-800-367-5296

Catalog # 017-000-011

Avidin Biotin Blocking Kit

Vector Laboratories, Inc.

Burlingame, CA 94010

www.vectorlabs.com

1-800-227-6666

Catalog # SP-2001

Primary antibody: Rabbit anti-phospho-Histone H2B (Ser14)

Upstate Cell Signaling Solutions

Lake Placid, NY 12946

www.upstate.com

1-800-233-3991

Catalog # 07-191

Negative control serum: Normal Rabbit Serum

Jackson Immunoresearch Laboratories, Inc.

West Grove, PA 19390

www.jacksonimmuno.com

1-800-367-5296

Catalog #011-000-001

Secondary antibody: Donkey anti-Rabbit

Jackson Immunoresearch Laboratories, Inc.

West Grove, PA 19390

www.jacksonimmuno.com

1-800-367-5296

Catalog# 711-065-152

Label: Vector Standard Elite Kit

Vector Laboratories, Inc.

Burlingame, CA 94010

www.vectorlabs.com

1-800-227-6666

Staining Procedure

Positive Control Tissue: Rat thymus (apoptotic bodies)

Stain Localization: Nuclear

Deparaffinize and hydrate slides through the following solutions.

Xylene	2 times	5 minutes
100% Ethanol	2 times	3 minutes
95% Ethanol	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 minutes. Temperature_____

Rinse in distilled water for 3 minutes. Repeat twice.

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

5. Block with 10% Normal Donkey Serum for 20 minutes at room temperature.

Lot#_____ Reconstituted Date_____

DO NOT RINSE SLIDES. CONTINUE TO AVIDIN-BIOTIN.

6. Apply Avidin/Biotin block

Lot#_____ Exp. Date_____ New Kit: yes / no

Apply avidin block - 15 min at RT.

Quick rinse in 1X AB.

Apply biotin block - 15 min at RT.

Wipe excess block

DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.

7. Apply primary antibody (Histone H2B (Ser14)) at a 1:1000 dilution and incubate for one hour at room temperature.

Lot# _____ Exp. Date _____

For negative control slides, normalize the protein concentration of the normal rabbit serum to the protein concentration of the primary antibody (Histone H2B (Ser14)) and use this to make a 1:1000 dilution and incubate for one hour at room temperature.

Lot# _____ Reconstituted Date _____

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

9. Apply Donkey anti-rabbit secondary antibody at a 1:500 dilution and incubate for 30 minutes at room temperature.

Lot# _____ Reconstituted Date _____

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

11. Apply Vector Standard Elite Label and incubate for 30 minutes at room temperature.

Lot # _____ Exp. Date _____

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

13. 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

14. Rinse in tap water 3 minutes.

15. Counterstain with Modified Harris Hematoxylin for 30 seconds.

16. Rinse in tap water until water is clear.

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

18. Dehydrate through the following solutions.

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

19. Coverslip

Updated 04/06/06