

Detection of phospho-Histone H3 (Ser10) in Formalin-Fixed, Paraffin-Embedded Human Tissue

Reagent and Antibody Information

[1X Wash Buffer](#)

[3% Hydrogen Peroxide](#)

[1% BSA Diluent](#)

[1X Citrate Buffer](#)

[Normal Rabbit IgG – Affinity Purified](#)

[DAB Chromogen](#)

[Hematoxylin](#)

Blocking Serum: Normal Goat Serum

Jackson ImmunoResearch Laboratories, Inc.

West Grove, PA 19390

www.jacksonimmuno.com

1-800-367-5296

Catalog # 005-000-121

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 H3 (Ser10) Antibody

Millipore

Billerica, Massachusetts 01821

www.millipore.com

1-800-645-5476

Catalog # 09-797

Secondary Antibody: Biotinylated Goat Anti-Rabbit IgG (H+L)

Vector Laboratories, Inc.

Burlingame, CA 94010

www.vectorlabs.com

1-800-227-6666

Catalog # BA-1000

Label Complex: R.T.U. Vectastain Elite ABC Reagent

Vector Laboratories, Inc.

Burlingame, CA 94010

www.vectorlabs.com

1-800-227-6666

Catalog # PK-7100

Staining Procedure

Positive Control Tissue: Breast carcinoma

Stain Localization: Nuclear

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 the slides in 2 changes of 1X wash buffer for 5 minutes each.

4. Heat-Induced Epitope Retrieval Using The NxGen Decloaking Chamber™

Add 500 ml of distilled water to the pan inside the decloaker. All three of the decloaker's containers must be filled. Any containers without samples should have 250 ml of distilled water. The samples need to be in a container with a full rack of slides and about 200 ml of **1X citrate buffer**. (Insert blank slides into any empty slots in the rack to ensure even heating of slides.)

Decloak the slides for 15 minutes at 110°C. *Maximum Pressure* _____

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. Block with 10% normal goat serum for 20 minutes at room temperature.

Lot # _____ Date Reconstituted _____

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

6. 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 BLOCK.

7. Apply primary antibody at a 1:500 dilution. Incubate for 1 hour at room temperature.

Lot # _____ Exp Date _____

For negative control slides, dilute normal rabbit IgG so that it's IgG protein concentration matches that of the primary antibody (if necessary). Then make a 1:500 dilution. If the concentrations can't be matched using this method, the dilution for the negative reagent may need to be adjusted. Apply the negative and incubate for 1 hour at room temperature.

Lot # _____ Exp. Date _____

8. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.
9. Apply the goat anti-rabbit secondary antibody at a 1:500 dilution. Incubate for 30 minutes at room temperature.
Lot # _____ Date Reconstituted _____
10. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each time.
11. Apply the Vectastain R.T.U. Elite Label and incubate for 30 minutes at room temperature.
Exp. Date _____ New Kit: yes / no
12. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.
13. Apply the DAB chromogen. 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
14. Rinse the slides in tap water 3 minutes.
15. Counterstain with hematoxylin for 20 seconds.
16. Rinse the slides in tap water until water is clear.
17. Gently agitate slides in 1X wash buffer until the tissues turn blue.
18. Dehydrate through the following solutions:

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

19. Coverslip

Updated 10/21/11